

What now for the Soviet Union?

Events in Lithuania in the past few days signal the end of the Soviet Union as it has been for the past few years. Whatever becomes of it, it will remain a nuclear power on a grand scale.

THE activities of the Red Army in Vilnius, the capital of Lithuania, at the weekend will cast a long shadow not just over the Baltic republics but over the Soviet Union as a whole. Mr Mikhail Gorbachev, whose uncharacteristic silence during these events is his final humiliation, is evidently also a party to the erosion of *glasnost* (the independent news agency Interfax was shut down last week, for example) and to the dimming of the hope — it was never, in reality, much more than that — of *perestroika*. It is a great misfortune that one so able should be so humbled.

The optimists in Moscow are now saying that they hope the seemingly inevitable civil war will be quick, much as are the military planners in the Gulf. Their assumptions are that what has happened in the Baltic states is a foretaste of what will happen elsewhere, perhaps even in the Russian Republic that stretches from the Ukraine to the Pacific, and that the Red Army's capacity to suppress it will be weakened by the same centrifugal tendencies, blunting soldiers' willingness to carry out distasteful orders. There are some grounds for that belief. For the time being, for example, the southern republic of Armenia is a no-go area for the centre's soldiers.

But that could quickly change. Everything will hang on the determination of whoever are the implacable men at the centre to wrest back control from the people who have dispersed it to the fifteen republic parliaments, and on their capacity to command the loyalty of their soldiers. The remarkable message from Vilnius on page 186 shows that the optimists in Moscow may be over-sanguine. It has become habitual to say that, with all the changes there have been in recent years, the old regime could never be restored. But that does not ensure that nobody will seek to try. There is much to fear in the months ahead.

There is also the question of Soviet nuclear weapons to keep the rest of us awake at nights. In the past six months or so, the Soviet government has been at pains to explain to governments in the West that it remained securely in central control of its strategic stockpiles. When people were alarmed that the growing independence of the republics might presage the emergence of as many independent nuclear powers, informal seminars were told by Soviet officials of the arrangements for controlling from the centre the use of Soviet nuclear weapons.

Now the difficulty is almost the obverse of that: whether the outcome is a loose federation of autonomous

republics or an autarchy (Leninist or otherwise), it will be a nuclear power on a grand scale. But not necessarily a superpower in the old sense. The economic weakness of what remains the Soviet Union, made generally apparent in the past few months, suggests that keeping pace with others in the development of delivery systems will not be feasible in the long run. So the worst outcome would be a repressive state replete with nuclear explosives, but one not easily brought within the ambit of international or even bilateral negotiations because of the truculence bred from insecurity. That is yet another reason for hoping that the optimists in Moscow are right. □

US squeamishness

The US government, by timidity over the use of fetal tissue, cannot discharge its ethical responsibilities.

IT IS, of course, admirable that the American Society of Obstetricians and Gynaecologists and the American Fertility Society have set up a committee to give advice to researchers on the ethical aspects of their work (see page 184). That the field bristles with difficulties is widely understood. And while responsible researchers in fields such as *in vitro* fertilization and the transplantation of fetal tissue are among the first to acknowledge what the difficulties are — and usually to define them in the first place — the public reputation of research requires that there should be some mechanism for telling the outside world that researchers habitually consider the ethical problems that confront them. The new board should help to serve that purpose in the United States. But, shamefully, it will cover only research supported by private funds, charitable foundations and the like.

The explanation is all too familiar: research of this kind is not supported by federal funds because the federal government shrinks from supporting it. Although this position is not quite the same as a declaration that all research of this kind is unethical, but is rather the consequence of political opposition to some potential applications of the research, the paradoxical consequence is that the federal government cannot exercise what might be considered its proper responsibility — that of arranging that all researchers are given ethical advice and even of requiring, if necessary by law, that they follow it. □



Prospects improve for US-European collaboration

- Joint US-EC task force to meet next month
- New framework for trade agreements

Washington

SCIENTIFIC relations between the United States and the European Communities (EC) are on the mend, when only ten months ago they appeared to be in a mess. Then, EC research commissioner Filippo Pandolfi had just left Washington with apparently empty hands after surprising White House science adviser D. Allan Bromley (and many of his own staff) with an unexpected proposal for a joint US-EC science working group.

US officials attracted by the idea were finding converts hard to win, with the EC science budget representing just four per cent of basic research spending by EC nations. And few were eager to tinker with traditional bilateral research agreements, which had been encouraged by the free-trade umbrella of the General Agreements on Tariffs and Trade (GATT).

But now the picture has been radically changed by Bromley's decision to embrace Pandolfi's proposal for a joint research policy task force (see *Nature* 344, 181; 1990). The new body, known as the US-EC Joint Consultative Group on Science and Technology, will have its first meeting in Washington next month, and intends to continue doing so every six or twelve months.

The increase of the EC research budget by more than 300 million ECU over five years and the collapse of the GATT talks last month have also helped to improve the outlook for research collaborations.

With Bromley's support (he has already set up a separate working group to coordinate EC research issues among the US agencies), EC science and technology seem to be rising quickly on the list of US priorities. Policy analysts say that, following the collapse of the GATT talks, the United States and the EC need a new framework for high-technology trade.

Research collaborations, with technology-transfer, competitiveness and intellectual-property protection built in, can provide a relatively uncontroversial model for new agreements. National Science Foundation (NSF) analyst Richard Bradshaw argues that if science and technology drive trade and international competitiveness, collaboration should make it possible to "leapfrog emerging trade barriers".

US officials are also encouraged to note that support seems to be growing in Europe for a stronger EC role in science and technology. Late last year, a report by NSF's National Science Board noted that

several countries, particularly the smaller European states, were in favour of an expanded role for EC, and that other member states, "until quite recently, ambivalent about sharing control", are being carried in that direction by "growing demands on national resources".

Although the body will not set up collaborations or manage them, it is intended to bring together US and EC agencies to set standards for joint research. There are at present about 15 *ad hoc* agreements between the United States and EC, mostly in fields such as fusion and nuclear safety, as well as more than 110 bilateral agreements between the United States and EC governments. How much and what kind of research should be handled at the EC, rather than national, level should provide continuing debate.

Five principal topics will be on the agenda of next month's meeting:

■ **Biotechnology.** Last September the United States and the EC set up a joint task force on biotechnology to coordinate scientific exchanges and cooperation. The group has already met; its progress will be reviewed by the Pandolfi-Bromley working group.

■ **Information Technology.** The group will discuss further expansion of US and European computer networks, as well as new arrangements for the storage and distribution of research data.

■ **Assistance to Eastern Europe.** Rebuilding the Eastern European research structure and helping Eastern European researchers remains a high priority for the EC. Although the United States has not yet played a significant role in that effort, the NSF is considering a pilot programme for science and technology aid to Eastern Europe.

■ **Human Resources.** Both the United States and the EC are concerned about the availability and supply of researchers. The working group, however, is not expected to discuss the potentially acrimonious issue of the 'brain drain' of European researchers to the United States.

■ **'Big Science'.** The clumsy US handling of the international collaboration aspects of big science projects such as the Superconducting Super Collider and the Space Station has been a favourite target for Bromley. Although those particular projects are probably beyond redemption, Bromley is hoping to build a framework for negotiations on future projects, such as the ITER fusion reactor.

Christopher Anderson

EC RESEARCH CENTRES

Early retirement spawns money row

London

THE European Commission's Joint Research Centre (JRC) has incurred the wrath of the the European Court of Auditors, the financial watchdog of the European Communities (EC), for wasting money on payments to staff retiring early from the centre.

More than 200 researchers, technicians and administrative staff have left the JRC since 1987 under two early-retirement schemes, which aimed to reduce the average age of JRC employees and recruit more scientists from the new EC member states of Spain and Portugal. But the annual report from the Court of Auditors says the schemes have failed in both regards. The report adds that the schemes were not targeted to improve the scientific quality of the JRC, relying on voluntary retirements, and that too many of the retiring staff were low-grade technicians, rather than research scientists.

Under the schemes, staff on five-year contracts terminable at only three months notice could receive 70 per cent of their basic salaries until the age of 65, the Court of Auditors says, estimating that this has cost more than 10 million ECU (about £7 million) up to the end of 1990.

JRC director-general Jean-Pierre Contzen rejects the criticisms. The first scheme, which began in 1987, was applied elsewhere in the EC, not just at the JRC, he says. Contzen says that it is important to rejuvenate the "infrastructure" of technicians as well as the JRC's research staff.

The new row follows a period of restructuring, completed in 1988, when the four old Joint Research Centres at Karlsruhe in Germany, Geel in Belgium, Petten in the Netherlands and Ispra in Italy were transformed into the single JRC, structured into nine institutes divided between the four sites (see *Nature* 338, 732; 1989). Ironically, the restructuring was prompted by previous accusations of poor management.

Glyn Ford, leader of the British Labour Members of the European Parliament (MEPs) and a member of the Parliament's Energy, Research and Technology Committee, describes the Court of Auditors report as "extremely damning". He singles out the Ispra site, which he says also has an extremely bad record at winning outside research contracts: "It's important that the EC spends more on high-tech R&D, but the activities of Ispra give that a bad name".

But fellow Labour MEP Gordon Adam, vice-chairman of the Energy, Research and Technology Committee, says that the JRC's apparent inefficiency is "by no means unique" among Commission bodies. The full effect of the retirement schemes on the average age of JRC staff will be seen only when all the replacements are made, says Adam.

Peter Aldhous

Spend more to compete

London

A NEW £10-million-a-year fund should be set up to help British universities meet the overhead costs of research projects supported by grants from the European Communities (EC), according to a cross-party House of Commons committee.

But the Education, Science and Arts (ES&A) Committee's report on the European dimension of British science policy says there is no need for a cabinet-level science minister to represent the interests of British science in Europe — an issue expected to loom large in a major inquiry into British science policy launched last week by the Royal Society (see below).

British academics appear to be successful in winning grants from the EC's SCIENCE programme for basic research, recovering more money than Britain contributes to the programme. The ES&A committee seems to have been persuaded by a succession of witnesses that the universities contribute more towards the indirect costs of European research projects than towards those supported by British research council grants. In most other EC countries, the overhead costs of EC research projects are met largely by individual governments, which puts British researchers at a disadvantage.

This claim was accepted by the ES&A committee, whence its recommendation of the new fund, to be run by the Advisory Board for the Research Councils. The committee would provide universities and polytechnics with an extra 20–30 per cent of the value of EC grants received, which would at present amount to £10 million of new money a year.

But if UK academics are disadvantaged in accepting EC grants by the burden of overheads, why do British scientists submit more applications to the SCIENCE programme than those from any other EC member state?

One reason may be that the British domestic science budget is too small, forcing UK academics to turn to the EC for support. The ES&A committee urges the government to increase its spending on civil science by 50 per cent, to match the proportion of gross domestic product spent in France and Germany.

This plea is unlikely to be accepted by the British Treasury, whose tightening of the public purse-strings has already precipitated a financial crisis for the research councils (see *Nature* 348, 377; 29 November 1990).

The ES&A committee's report also attacks the Treasury's practice of 'attribution' — that of subtracting a proportion of the money won by British scientists from EC research programmes from the following year's domestic spending on science. In this respect, the

ES&A committee echoes the earlier criticisms of the House of Lords EC committee (see *Nature* 346, 305; 1990).

Many British scientists hold that the UK scientific community has too little influence when EC research programmes are drawn up. Britain is represented at meetings of EC research ministers by an industry minister, currently Lord Hesketh. Although briefed by officials from the Department of Education of Science (DES), some of the committee's witnesses argued that an industry minister could not adequately represent British academics.

The report says that the DES minister responsible for basic science, currently Alan Howarth, should also attend meetings of EC research ministers, but says

UK SCIENCE POLICY

Royal Society announces policy inquiry

London

THE Royal Society announced last week that it is to carry out a wide-ranging inquiry into the conduct of science policy in Britain. The inquiry could be particularly influential, coming as it does so

that a separate cabinet minister to co-ordinate British science is not necessary. Indeed, the committee argues that a new ministry of science would be inappropriate, cutting across the need that most UK government departments should also have a strong science base. The committee also says that a minister with merely a co-ordinating function and no departmental budget may lack real influence over policy decisions.

The debate over the value of cabinet representation for British science, which has intensified since the departure of Margaret Thatcher as prime minister, will not be silenced by the ES&A committee's report. The Save British Science pressure group has made the appointment of a cabinet-level science minister one of its targets. This issue is also likely to feature in the Royal Society's inquiry into British science policy.

Peter Aldhous

public before then.

Among other things, according to Brian Follett, biological secretary of the Royal Society, the inquiry will probably weigh the advantages and disadvantages of a cabinet-rank minister. Follett would like

to see cabinet representation for science, provided that the minister concerned is given a suitable agenda.

But Sir Francis Graham-Smith, the society's physical secretary, remains unconvinced, unless a cabinet appointment is the only way to achieve reform of the damaging practice of attribution for EC research spending against the Department of Education and Science science budget (see above).

The Royal Society's inquiry will encompass most aspects of UK science policy, and has been prompted by a number of changes over the 1980s — including the increasing proportion of researchers on short-term contracts, the declining proportion of research financed by government and the expansion of international collaboration.

Peter Aldhous

■ The meeting between Prime Minister John Major and a delegation of prominent British scientists, planned for 14 January, has been postponed because of the crisis in the Gulf. The delegation, led by Conservative MP Sir Ian Lloyd, is expected to argue for a new deal for British science, including representation in the cabinet. □



Sir Michael Atiyah — directing the inquiry.

soon after the change of prime minister and at a time when British connections with the European mainland will be further strengthened after 1992.

The work will be directed by a team of eight fellows of the Royal Society under the chairmanship of Sir Michael Atiyah, president of the society since the end of last November. A final report of the inquiry is expected sometime in early 1992, but findings on specific issues may be made

New panel for ethical issues

Washington

FRUSTRATED by the US government's failure to provide the proper national forum for public debate, review and oversight of ethical issues in biomedical research, two medical organizations announced last week that they will establish a national advisory board to set ethical guidelines in reproductive and fetal tissue research.

The new 15-member board, to be known as the National Advisory Board on Ethics in Reproduction, will consist of lawyers, students of ethical issues, theologians, scientists and members of the public. The board aims to set guidelines in the sensitive areas of reproductive and fetal tissue research and will provide peer review of research proposals.

Because the board will have no legal authority, compliance with its recommendations will be voluntary. Financial support for its work will, to begin with, be provided by the two founding medical societies, the American College of Obstetricians and Gynaecologists (ACOG) and the American Fertility Society (AFS). Federal support for research involving *in vitro* fertilization and embryo transfer has not been available since 1980, when the Reagan administration allowed the Ethical Advisory Board (EAB) to the Secretary of Health and Human Services (HHS) to lapse. The administration's position has not changed since 1980.

A temporary ban on the federal funding of fetal tissue transplantation research in humans also took effect in March 1988, and was extended indefinitely in November 1989 by HHS Secretary Louis Sullivan. That decision, taken against the advice of an *ad hoc* NIH panel on the ethical issues of fetal transplantation, was based on the view that permitting such research would increase the incidence of abortions.

The new board will function most immediately in relation to privately funded research proposals, which are at present reviewed at a local level by institutional review boards, where they exist. Howard Jones, AFS spokesman and professor of obstetrics and gynaecology at the Eastern Virginia Medical School, says that local boards, when facing tough ethical questions, "sometimes feel they need consultation".

In a written statement, Kenneth Ryan, ACOG spokesman and chairman of the department of obstetrics and gynaecology at Harvard Medical School, argues that research on fetal tissue and reproductive technology is sustained and will continue "with or without government regulation", and that "the time is ripe for a private group to shoulder the task of setting stan-

dards" to ensure that it is scientifically and ethically sound.

Predictably, the new board was denounced by Douglas Johnson, legislative director of the National Right to Life Committee, who sees it as an attempt to erode public support for the pro-life policies that are in place. "All of these matters involve some pretty fundamental ethical concerns, and these should be decided through the democratic policy mechanisms which exist and not by a tiny clique of people who have an axe to grind", says Johnson.

The founding organizations hope that the members of the new board will be elected within six months.

Fetal tissue transplantation research offers promise for patients with neurological disorders such as Parkinson's and Alzheimer's disease. Several Parkinson's disease organizations and the Association of American Medical Colleges propose to challenge the legality of the administration's ban on the use of federal funds in a US federal district court.

A Chicago-based law firm has agreed to take on the case in the public interest. Dinah Tottenham Orr, executive director of the Parkinson's Disease Foundation, says that other disease groups may well follow suit.

Diane Gershon

UK SUPERCOMPUTING

London centre keeps Cray

London

A row that had threatened the continued existence of the University of London Computer Centre (ULCC), one of only three British national centres for academic supercomputing, seems to have been resolved in a compromise that will transfer a smaller Amdahl machine from ULCC to the University of Manchester, where costs are lower.

The threat to ULCC arose last summer, when the Computer Board of the Department of Education and Science (DES) ruled that the cost of running the centre's Cray supercomputer and Amdahl machine, both of which are intended as national facilities, should be reduced from about £3.3 million to £1.5 million a year. This would have brought ULCC's running costs into line with the similar-sized Manchester centre.

The University of London rejected this plan, which would have saddled it with more of ULCC's running costs. This is already the practice at Manchester, where the national supercomputing facility is run at marginal cost alongside the local centre. But the court of the University of London seems to have argued successfully that circumstances in London are different. Each

Nature appointment

London

BARBARA J. Culliton has been appointed deputy editor of *Nature* with effect from 1 February. Ms Culliton has been a member of the editorial staff of the AAAS journal *Science* since 1972, during which period she worked as news editor for the decade until 1989.

Ms Culliton has a distinguished record in science journalism, having served as president of the US National Association of Science Writers (1981-82) and of the US Council for the Advancement of Science Writing (1985-89). In 1989 she was elected a member of the Institute of Medicine, and is one of only three journalists so honoured.

The Editor of *Nature*, John Maddox, says that Ms Culliton's appointment will further and excellently strengthen *Nature's* position in the United States, which accounts for more than 40 per cent of the journal's international circulation, now in excess of 50,000. He says that, apart from her plans to contribute to *Nature*, Ms Culliton has a number of exciting plans for strengthening the journal's service to the scientific community in the United States.

Ms Culliton will also be in administrative charge of *Nature's* US office in Washington, DC, where the journal's news-gathering is coordinated, and will be a senior partner in the evolution of editorial policy. □

of London's colleges has its own computer centre, funded separately from ULCC.

ULCC staff had feared that both machines would be transferred — the Amdahl to Manchester and the Cray to the larger Atlas centre at the Science and Engineering Research Council's Rutherford Appleton Laboratory.

Under the deal negotiated between the Computer Board and the university, ULCC's Cray-XMP will be replaced by a new machine, probably a Cray-YMP, which has a larger memory and is cheaper to run. Fewer than 20 ULCC staff are required to run the scalar computing service provided on the Amdahl, and the university expects that there will be no forced redundancies. The slimmed-down ULCC will cost about £2 million a year.

The University of London is pleased with the new arrangement, but British academics will still be left with poorer provision for supercomputing than many of their European colleagues. France and Italy each have almost twice the academic supercomputing power of Britain, and most German *Länder* have their own academic supercomputing centres supported in part with their own funds.

Peter Aldhous

Bureaucracy denies grants

Tokyo

MANY Japanese researchers are, for practical purposes, being denied access to funds available from the country's imaginative Human Frontier Science Program, based in Strasbourg, France. This came to light last week, in a further illustration of how Japan's bureaucracy can shoot its own ambitions in the foot.

In the mid-1980s, researchers belonging to the Ministry of International Trade and Industry (MITI) helped to formulate the programme, which supports international research on the brain and molecular biology. At that stage, they expected themselves to benefit from the programme. But it has now emerged that MITI researchers and thousands of others at national research laboratories are in effect barred from receiving Frontier grants by extremely rigid bureaucratic regulations.

One result is that there has been a dramatic fall in the number of applicants for Frontier grants from Japan's national laboratories in the latest round of applications, completed last September.

This is not the first time that such problems have come to light. Last year, researchers from Japan's national universities who were awarded Frontier grants were dismayed to find that, under government regulations, they had to pay about 50 per cent personal income tax on their awards. After months of wrangling between the government ministries and agencies, Frontier grants were eventually declared tax exempt under a special regulation by the Ministry of Finance (*Nature* 347, 703; 1990).

But Kenji Goto, deputy director of MITI's Frontier office, now says that "this was only the first chapter". The new Ministry of Finance regulation may have solved the problem for researchers at universities, but there is no mechanism by which those at national research laboratories can receive Frontier grants.

For researchers at national laboratories, Frontier grants would first have to go into a general account for all of Japan's ministries and government agencies and then be fed indirectly to the national laboratory concerned.

The rigid limits on ministry and agency spending decreed by the Ministry of Finance make this impractical. A recipient ministry would be able to receive its grant only by making cuts elsewhere in its spending. The confusion is especially ironical because the general account is also that from which general support for the Frontier programme is paid.

Goto and his colleagues at MITI's Agency of Industrial Science and Technology (AIST) have been labouring on this problem for the past two years. On 18

June last year, they issued a directive to AIST researchers suggesting that those seeking Frontier grants should organize their international research teams as "separate legal entities". Successful applicants would then be able to carry out "joint research" with the new entity. No money would change hands, but researchers would be allowed to "borrow" hardware bought with Frontier grants.

Some researchers are dismayed by this advice. Youji Mitsui, head of the Cell Science and Technology Division of AIST's Fermentation Research Institute in Tsukuba who, together with Tomoh Masaki of the University of Tsukuba, has won international recognition for pioneering research on endothelin peptides (see, for example, *Nature* 348, 673; 1990), has decided it would be a waste of time for him to apply for a Frontier grant.

He says that he does not need hardware, but chemicals, reagents and salaries for assistants. He also says that setting up an international research team composed of people from several different countries as a "separate legal entity" would cause tremendous headaches and a great deal of embarrassment if it failed.

Mitsui is not alone. Goto says that 15–20 people from Japanese national laboratories applied for Frontier grants in

the first round of applications in 1989, but that the number plummeted to "two or three" in 1990.

Goto and his colleagues at MITI realize that their solution, which tries to work within the present system, is by no means ideal. The real need is for a complete overhaul of the mechanism of research funding at Japan's national research institutes to create more flexibility. But that would forbiddingly require coordination of all ministries and agencies involved, including MITI, the Science and Technology Agency (STA), the Ministry of Health and Welfare, the Ministry of Agriculture, Forestry and Fisheries and the Ministry of Posts and Telecommunications.

STA is the lead agency for the Frontier programme, contributing 60 per cent of the cost. But STA officials, who would have to push for change, are reluctant to admit that there is a problem.

Ryuji Shimoda, director of STA's Frontier office, says that researchers at national institutes may have been discouraged from applying in 1990 by the intense international competition for Frontier grants, poor English ability and by the example of the 20 or so applicants in 1989, all of whom failed. He accepts that payment problems may also matter, but says that because no researcher from a national laboratory has yet won a Frontier grant, STA has "not yet looked into the matter in detail".

David Swinbanks

One tap is closed, but another is opened

Tsukuba

RESEARCHERS at MITI's Agency of Industrial Science and Technology (AIST) may be shut out from the Human Frontier Science Program, but they are now eligible for grants from a new domestic grant scheme modelled after Frontiers. The programme, run by AIST, was introduced in 1988, and is the first in Japan to have a western-style peer review system.

The grants, which are for research on "biological functions", are small, ranging from 3 to 13 million yen (\$23,000–\$100,000) a year for three years. But researchers can hold more than one grant simultaneously. There are about ten awards each year.

Youji Mitsui, of AIST's Fermentation Research Institute in Tsukuba, currently has three grants which help support a team of about 30 researchers and technicians drawn from his institute, universities and industry. Using an immortal endothelial cell line, the group is investigating the effects of endothelin on smooth muscle contraction, the recognition of cancer cells by endothelial cells in the walls of blood vessels, and gene expression of vasoactive intestinal contractor (VIC), a peptide very similar to endothelin that was discovered by Mitsui's group.

Similarly, a group led by Shigeru Yamane, chief of the Neuroscience Section of AIST's Electrotechnical Laboratory in Tsukuba, has more than one grant to investigate facial and colour recognition in the monkey brain and to store a complete map of a monkey brain in a Macintosh computer in the form of magnetic resonance images and histological sections.

Hiroshi Uemura, senior researcher of AIST's National Chemical Laboratory, who helped formulate the new grant programme, claims that it is unique for AIST because grant applications are screened by scientists both within and outside AIST, and not by government officials. Furthermore, rejected applicants receive an explanation for why their applications are turned down, something normally unheard of in Japan.

Because of the recent fluctuation in the yen-dollar exchange rate, AIST has been able to boost the budget for the programme in 1991 by nearly 20 per cent to ¥288 million (\$2.2 million) by drawing on funds originally intended for the Frontier programme. Even so, AIST researchers say that the budget needs to be at least doubled to have a significant impact. D.S.

Old secrets on the market

San Francisco

As Cold War tensions evaporate and the Soviet Union flounders economically, the Soviet Union has begun selling its technology, even sensitive technology with military potential, in the West. Last week, a California entrepreneur completed negotiations for the sale to the US government, at a price of \$10 million, of an advanced Soviet nuclear reactor for use in space. Just two years ago, even the reactor's existence was a closely guarded secret.

The device is a Topaz II reactor designed to power satellites and space stations. Its design is different from and more advanced than those under development in the United States, according to Joseph Wetch of San Jose, California, who orchestrated the agreement. The high-power system may have a variety of applications, including powering communications satellites, radar systems and even the first manned lunar outpost. The reactor is 12 feet high, six feet in diameter and weighs one ton. Its lifetime is estimated at five years.

For the United States, the advantages of the Topaz II reactor is that it uses thermionic rather than thermoelectric conversion to obtain electricity from the reactor heat. As designed, the system can generate up to 6 kilowatts of power, and is

probably more economical than solar cells when such a large output is required.

Wetch, who has spent two years negotiating the reactor agreement, believes there remains plenty of untapped science and technology in the Soviet Union. Obtaining it, however, is another matter, requiring good relationships with Soviet scientists and considerable patience with government bureaucracies. Chief among the obstacles were the high US import duty and long delays in the US Commerce Department.

Wetch has a special interest in nuclear space reactors, having been the principal developer of the first, known as the SNAP-10a, in 1965. Shortly after the reactor's first use in space flight, the budget of the US research programme was squeezed, and has been almost dormant for 15 years. The Soviet design now acquired may be able to provide power for space systems at lower costs than solar cells or liquid-fuel systems now in use. The Topaz II will be demonstrated and tested initially in Albuquerque, New Mexico, by a consortium of researchers from academic, industrial and national laboratories, using a Soviet electrical heater rather than the nuclear fuel used in orbit.

Meanwhile, Wetch is working on several other agreements through a joint venture known as INERTEK created

between his company, International Scientific Products, and Soviet research organizations. He believes that electronics technology for space and ceramic and metallurgical technologies will be among the fertile areas for future Soviet technology transfers. **Elizabeth Schaefer**

OPEN LETTER

A message from Vilnius

14 January 1991

On Monday this week, *Nature* received the following plea from scientists in Estonia, addressed to the worldwide scientific community:

"At this moment, when Soviet armed forces, starting in Lithuania, and with no respect for human life, are attempting to eliminate democratic structures, freely and rightfully elected parliaments and their appointed executives, we wish to state the following:

The West must acknowledge that the traditional liberal Western view on the future of the Soviet Union and its influence on world peace and stability is now out of date. A more realistic assessment is needed, based on the long history and traditions of the Soviet brand of imperialism. These can be traced back to the Civil War, the occupation and annexation of independent states in 1939 – 1940, to Hungary in 1956, Czechoslovakia 1968, Afghanistan and to more recent events in Tbilisi, Baku and elsewhere.

Those who support this imperialist ideology in the USSR, sacrificing half the national income to the military industrial complex, are today set on getting compensation for the loss of the 'Socialist camp'.

The only way in which the Western democracies can withstand the rising anti-perestroika tide and its neo-imperialistic attitudes is by open and clear support, not only of individuals but also of the democratically elected legislative bodies of the republics. Western governments should look behind the rhetoric and decide which carries the most promise for peace in Europe and elsewhere: a unitarian empire with constant lapses into brutality, or a democratic community of free states, the Russian federation among them.

We ask all scientists to persuade their governments to take a strong and unequivocal position in supporting Lithuania, the other Baltic states and freedom-seeking republics of the entity still referred to as the Soviet Union."

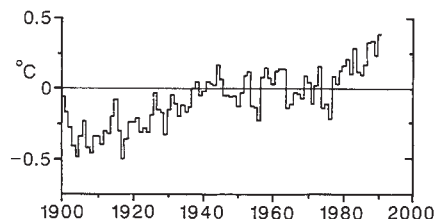
The message is signed by Professor Richard Wellems, director of the Estonian Biocentre, who is also a member of the praesidium of the Estonian Academy of Sciences. He says that he writes on behalf of scientists of the Estonian Academy of Sciences and universities, the Estonian Union of Scientists, Estonian Science Council and the Estonian Science Fund Council.

CLIMATE CHANGE

1990 warmest year on record

London

THE Earth's surface was, on the average, warmer in 1990 than in any year since records began in the mid-nineteenth century. The 1990 value for global mean surface temperature, released last week by the



Global mean surface temperature since 1990, relative to the mean from 1951–80.

UK Meteorological Office and the University of East Anglia (UEA)'s Climatic Research Unit, exceeded the previous record, in 1988, by 0.05 °C. But it is still too early to say with certainty whether the recent warmth is due to the build-up of greenhouse gases in the atmosphere.

Six of the seven warmest years on record have occurred since 1980, making the past decade the warmest ever recorded. The UEA team is examining the link between

the observed warming and the concentration of greenhouse gases in the atmosphere. The global mean surface temperature is too simple a measure to be of any use, so the UEA researchers are working with temperature values for each of the squares of a worldwide grid, comparing the data over the past century with predictions from computer models of warming under the greenhouse effect. "We think we're beginning to see a link", says Phil Jones, from UEA.

Jones says that the stratosphere appears to be cooling, as predicted by the greenhouse theory. But there are some minor discrepancies: the stratospheric cooling is slower than expected, and surface warming is greater at mid-latitudes and less at the poles than in computer simulations of greenhouse warming.

The Meteorological Office/UEA value is the most reliable calculation of the global mean surface temperature, based on data from land- and sea-based monitoring stations. A similar value produced by the NASA Goddard Institute for Space Studies is calculated from land-based data alone.

Peter Aldhous

AIDS politics change rules

San Francisco

In the next two or three months, the US Food and Drug Administration (FDA) is expected to begin review of two promising new AIDS drugs, the first such materials to be entered into the approvals procedure since AZT was approved four years ago.

This development will be a landmark test of procedures for the evaluation of new AIDS drugs. In the past few years, the worsening AIDS epidemic has brought unprecedented pressure on the FDA to approve new drugs more quickly. The FDA will now have a chance to show AIDS activists and others how quickly it can respond, but its handling of these two new compounds may also indicate how much AIDS has changed the politics of health care in the United States.

Both drugs — dideoxyinosine (ddI) and dideoxycytidine (ddC) — have shown promise in clinical trials, but both can have serious side-effects. What constitutes adequate proof of their efficacy has never been completely defined by the health officials who will have to rule on them.

That imprecision, say advocates of the interests of AIDS patients, is delaying the process of gaining market approval for the treatments. And for the gravely ill, for whom the only approved AIDS drug, AZT, causes intolerable side-effects or is no longer effective, the delay, they say, costs lives.

The FDA will very soon have to face the issue of which criteria are acceptable for the evaluation of ddI and ddC. The manufacturers say they still have to complete some data analysis before they can file for FDA approval — a process that will take from weeks to months or more.

But even if the FDA received approval requests from the pharmaceutical companies today, it would be unable to respond, says David Barr, of the Gay Men's Health Crisis in New York, in the absence of established guidelines for the approval of AIDS antivirals. This "can only retard the development of new drugs", according to a formal petition filed with FDA last month by the Community Consortium, an association of 180 San Francisco Bay Area health-care providers who treat most of the people with human immunodeficiency virus (HIV) in the region.

Donald Abrams, professor at the University of California at San Francisco and chairman of the consortium, says that the FDA should also say what it looks for in the approval process to help defuse the current debate about the adequacy of the data available from the trials so far carried out.

Although they are expected to file for

approval soon, both companies, Bristol-Myers Squibb, which makes ddI, and Hoffmann-La Roche, which makes ddC, want to carry out further tests that could take years. But activists say the data are already adequate and are imploring the companies to hurry their analysis and data submission.

Meanwhile, activists and some researchers hope that a turning point in the development of clear-cut guidelines will come on 13 February, when FDA's Antiviral Drugs Advisory Committee meets to discuss what endpoints or standards will be acceptable proof of efficacy. The Community Consortium, in its letter to FDA Commissioner David Kessler pressing for rapid approval of ddI and ddC, asserts that the government has authority to demand that the pharmaceutical companies should submit their current data by 1 February, and could resolve to decide the fate of ddI and ddC by 1 March. FDA has no comment yet on this point.

FDA's discussions will also be affected, and perhaps slowed, by the loss of Ellen Cooper, who resigned because of the pressure of work last month, after three years as the director of the Division of Antiviral Drug Products. Last week, the FDA announced that the position will be held temporarily by Carl Peck, Cooper's former superior, who directs the Center for Drug Evaluation and Research. Activists were widely disappointed at the departure of Cooper, whom they considered something of an ally within the administration.

Meanwhile, thousands of the estimated one million people in the United States infected with the AIDS virus are taking ddI or ddC through clinical trials and "expanded access" programmes. But activists say those procedures fall far short of the need. Expanded access, for example, makes promising drugs available only to patients for whom AZT's effectiveness has diminished or who cannot tolerate it, and excludes those who cannot afford a primary care physician or who do not meet specific health criteria whatever their physicians recommend.

Because it is considered unethical to use placebos in studies of people with the life-threatening disease, data from controlled trials are not available, while the use as controls of patients from earlier studies is complicated by the changed treatment of AIDS over the years. Now, for example, pentamidine is widely used to treat the common opportunistic pneumonia, known as PCP, to which AIDS patients are prone, while the strains of HIV infecting patients have also evolved.

Studies therefore focus on comparisons of the effects of new drugs with those of AZT, which also raises complications.

Does it suffice for a drug to be as effective as AZT, or must it be better? Further complicating the picture is evidence indicating that ddI and ddC are most effective when they are used in combination with AZT.

Some argue that in the evaluation of new drugs, more reliance should be placed on their effects on biochemical features known as "surrogate markers" — the numbers of key immune cells in the patient's blood or the amounts of viral antigen present, for example. In AIDS, the most promising surrogate marker is widely thought to be the blood concentration of the immune cells known as CD4, which are the principal targets for attack by HIV.

The notion that increased concentrations of CD4 cells are an index of improved health is supported by most, but not all, studies so far carried out. But critics argue that CD4 cells are an imprecise index of the health of the immune system, and may not indicate real cause-and-effect. Similar concerns have been raised about measurements of the p24 antigen of HIV, which reveals the level of actively replicating virus in the body.

Stanford University AIDS researcher Thomas Merigan believes that these surrogate markers would nevertheless be reliable in comparisons of the effects of ddI or ddC with those of AZT, on the grounds that all three drugs function by the same mechanism.

Deciding on the acceptability of surrogate markers is among the most crucial issues in AIDS politics today, says Mark Harrington of ACT UP, the AIDS Coalition to Unleash Power, in New York. Scientists and activists promise to watch closely the outcome for ddI and ddC, believing that the result may determine the fate of the new classes of AIDS pharmaceuticals on the horizon.

Elizabeth Schaefer

RESEARCH FOUNDATIONS

First woman director for Wellcome

London

BRIDGET Ogilvie is to be the next director of the Wellcome Trust, Britain's largest medical research charity, becoming the first woman to head the organization. She replaces Peter Williams, director since 1965, who retires at the end of September. Ogilvie was formerly a parasitologist at the Medical Research Council's National Institute for Medical Research in London.

The Wellcome Trust was set up in 1936 under the will of Sir Henry Wellcome, founder of the Burroughs Wellcome pharmaceutical company. The trust will this year spend £78.7 million on medical research and the study of the history of medicine.

Peter Aldhous

On the margins of Europe

SIR—Your leading article (*Nature* 348, 375; 1990) catalogues the decline in the fortunes of British research and higher education but does not address the fundamental issue of whether the Britain of the 1990s will get value for money from its expenditure on these activities.

There are two justifications for research funding: (1) investment leading directly to innovations of commercial value; (2) training individuals who will do innovative research in industry. Both justifications assume that Britain contains numerous technology-based companies capable of benefiting from this investment. We have only to look around us to see that this is untrue. Britain acts as an offshore manufacturing base for companies that carry out their research and development elsewhere, while independent British manufacturers service these companies with low-technology products that are cheaper to manufacture here than overseas. Economic growth in the past decade has been concentrated in the service sector, which is not renowned for its interest in leading-edge technology. People are making excellent livings from these activities and they are entitled to ask why they should spend their money on research that does not enhance their prosperity.

This argument extends to the whole of higher education. The shift to entrepreneurial values means that the skills engendered by the higher education system are not high on the agenda of employers, who recruit graduates because the intellectually ablest school-leavers insist on entering higher education, but who both despise the values embodied in higher education and bitterly resent the costs to them through taxation.

Our neighbours recognize that prosperity comes through advanced, high-value-added products that can be manufactured only in countries with the necessary intellectual infrastructure. Previous British administrations have recognized this but not the importance of encouraging commercial initiative. The present administration has supported individual enterprise but has undermined the infrastructure while still spending heavily on it. Present patterns of employment, as encouraged by the government, do not require either state-funded research or a massive higher education system, so the government should have the courage of its convictions and eliminate, once and for all, the enormous tax burden they entail and divert the money back to employers for training. The urgent question is whether current levels of prosperity can be sustained on this basis given that the bubble of the service sector has already burst, with disastrous consequences, and our attractiveness as an offshore manufac-

turing base is threatened by competition from our European neighbours. If it genuinely wants Britain to remain prosperous into the next century, the government might consider putting enough money into research and higher education to prevent their physical disintegration and the total demoralization of their staff. Failure to do so will marginalize Britain in Europe more surely than the histrionics of any single member of the cabinet.

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Who speaks for science?

SIR—There has been extensive reporting in your columns in recent years about the declining plight of British science and the declining morale of British scientists, and the most active body publicly lobbying support for the British scientific base seems to be Save British Science. It must surely be asked what is the position of Royal Society? This is the traditional body of eminent scientists who could speak with real authority on the causes, consequences and solutions to the current situation. The Royal Society should be the voice to which British scientists, both at home and abroad, would turn for leadership; unfortunately, the voice seems mute.

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■ See News, page 183.

Soul not for sale

SIR—In response to Philip Siekevitz's letter "Science sells its soul" (*Nature* 347, 509; 1990), as a young researcher I disagree with his assertion that commercialization of research by university departments is an abhorrent corruption of their founding philosophy. UK science — biomedical in particular — is in a cash crisis. The ultrapurist allure of science he describes belongs to an almost utopian era when sufficient funds existed for science projects to be pursued irrespective of cost. This is a mythical assumption based largely on a British imperial legacy of "gentleman scientists".

Entry into science was and still is based on this popular press image. My soul and motivation are still driven by the allure of which he speaks. For example, I have for

three years been a weekend shopworker at Marks & Spencers — as well as a weekend research fellow. However, I now embrace links with money-making biotechnology companies with open arms. This may seem to him to be selling my soul but I believe it is a realization of the current facts of life (if not science).

Nature has frequently reported the shortage of government cash coming from the Medical Research Council. This is further reflected in a reduction of grants from the cancer charities. It is becoming increasingly difficult, if not impossible, for newcomers to gain grants in competition with established large research groups, especially now that even these are being cut (*Nature* 347, 324; 1990).

Courting biotechnology companies is the only alternative. It provides, for me at least, a possible source of cash for a newcomer. It helps to fund and expand the research to which the allure of science has made me an addict. Making discoveries, pursuing chance observations to their conclusion, and, of course, being recognized for the scientific endeavour, is still my prime motivation. However, I will advise, produce methodologies, contract for research, evaluate products and sell purified proteins willingly to any biotechnology company.

Sir, I have not sold my soul, but I will prostitute my principles to keep my soul alive.

RAY ILES

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Did anyone know Frederick Soddy?

SIR—I am writing a biography of Frederick Soddy, 1877–1956. He collaborated with Rutherford on the very early radioactive research at McGill in Montreal in 1903–04 and was awarded the Nobel Prize for his work on isotopes. He was at the Universities of Glasgow, Aberdeen and finally Oxford until his early retirement. During the latter period he had the reputation of being something of a crank because of his interest in monetary reform.

I would be very interested to hear from anyone who knew him or knew of him or can offer any suggestions for sources of information about him.

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Making global warming public property

There is a long way to go before the research community concerned with the behaviour of the Earth's atmosphere will win general acceptance of its message that global warming is serious. Less zeal and more guile would help.

THE Ditchley Foundation, the British charity whose chief asset is the right to use a splendid tudor manor house in Oxfordshire as a conference centre, has embraced global warming. Last weekend, it invited a group of people from mixed backgrounds (roughly a third each from Britain, the United States and elsewhere — mainland Europe and Japan) to make sense of this vexed issue. The chairman was Sir Crispin Tickell, until last summer the British Ambassador to the United Nations, now the Master of Green College, Oxford, who has done more than most people to put global warming on the international agenda. The meeting had been timed closely to precede the intergovernmental conference that opens in Washington on 4 February, at which a start will be made on the negotiation of a greenhouse convention.

What can a gathering like this suggest about the likely outcome of the eighteen months of negotiations that begin next month? Chiefly, that the way ahead is bound to be difficult. Even among a small group of people self-selected by earlier declarations of interest in the field, perspectives are so different as sometimes to seem orthogonal to each other.

How much more difficult must it be to win agreement among government delegations representing diametrically opposed interests? Some dispute the science of the problem. Some favour adaptation to climate change, and others its avoidance. Some developing countries may (as foretold last weekend) assert that global warming is a story told by the industrialized rich to prevent the development of the rest of the world.

What follows is an impression of an enthralling, but also admonitory, meeting; the rules of these occasions forbid ordinary reporting, in particular the attribution of even the most arresting statements to those who made them.

First, there is a semantic question: if the predictions of the trend of average temperature generated by the computer models of global climate are always attended by large uncertainties, what credence can be given to the simple story of global warming? In particular, if the uncertainties are so great, might it not be that extra carbon dioxide could lead to global cooling? Plainly it will not be easy to persuade the world at large that the greenhouse effect that has, since at least the Precambrian, made the surface of the

Earth habitable, is certain to work in the same direction when the concentration of greenhouse gases is artificially increased.

The modellers themselves exude a sense of injury in this connection. When the case is so clear, why are the sceptics so generously given a hearing? This is not just a matter of public relations (however nicely that phrase is meant), but of the attention and criticism people's work is given when, perhaps against their expectations, it turns out to be potentially of great practical significance. Those who happened, in the 1950s, to be working on the genetic effects of small doses of ionizing radiation quickly learned this lesson, often with chagrin. There seems to be a danger that at least some climatologists have not yet learned that those who question their conclusions, even without experience in the field, are not necessarily fools. (Others, it must be said, have learned to be as judicious as the Sphinx.)

Especially because the economic consequences of global warming are so great, as measured by the costs either of avoidance or adaptation, there is a case for asking that the modellers should go out of their way to give the sceptics a patient answer. If somebody argues, for example, that a more powerful greenhouse will, by increasing the circulation of water vapour in the lower troposphere, make the atmosphere dryer at greater altitude and thus remove water vapour (a greenhouse gas) from the region at which it is most effective, he should be given a more courteous hearing than is now usual.

It would also strengthen the case of the climatologists if people who model complicated systems other than the Earth's climate were given access, as constructive critics, to the models on which current predictions are based. Perhaps the outstanding precedent is that of the British Treasury which, almost two decades ago, made its economic model available to independent economists, many of them sceptical of its performance. Many who came to scoff were, as a consequence, converted. More practically, it is entirely possible that the next generation of computer models of the Earth's climate would be developed more effectively if there were a more vigorous exchange with those working in other fields.

What does appear beyond dispute is that the moderate case for the global warming story has been badly served by the manner in which it has been told.

Some would say that too much has been published, but that cannot by definition be true. But it is significant that documents that should have been influential have often been published so untidily that even sympathetic readers have been dismayed by them.

This applies to last summer's reports from the Intergovernmental Panel on Climate Change (IPCC), which made their way into print as successive drafts, often as drafts of what are called "executive" summaries. By all accounts, the report on which the US National Academy of Sciences has been working for the past eighteen months, and which had been intended for publication before next month's meeting in Washington, will avoid these difficulties even if its publication is delayed until after the conference is over.

The missing ingredient is a means by which even the research community can make sense of the original research related one way or another to global warming that now sees the light of day. The ideal would be a continuing authoritative review of the original literature. There is a precedent of a kind — the occasional volumes put out by the United Nations committee called UNSCEAR (the Scientific Committee on Energetic and Atomic Radiation), which have been powerful reinforcements of the radiation safety standards. But because some such assessment mechanism will be needed if ever there is a greenhouse convention, the sooner it is set up, the better.

Sadly, the same device would not suffice for the more difficult questions that will have to be answered in the years ahead. Global warming will affect not simply physical and biological systems (sea level and agriculture, for example), but the whole fabric of society. But who, at this stage, would guess at the extent to which substantially higher costs for surface transport will change the character of industrialized societies, and affect their productivity? Or how far an effective greenhouse convention will require that the world's population should also be regulated, and how? These, it should be acknowledged, are the real uncertainties, and the true impediments to decision. Sadly, the global warming community is over-zealous in its conviction that, with the physics of the atmosphere reasonably well understood, other difficulties should be swept aside in the pursuit of agreement.

John Maddox

Pruning the Lyman- α forest

John Peacock

ASTRONOMERS have spent the past decade using distant quasars as searchlights to reveal the nature of the very first galaxies. By studying the absorption lines seen in quasar spectra, we can detect the presence of objects on the line of sight which are far too faint to be seen via their own starlight. Although the information obtained in this way is incomplete in many tantalizing respects, a consensus about at least some of the properties of the absorbing gas did emerge: the gas seemed to occupy low-density, highly ionized 'clouds' of roughly galactic dimensions¹. All that may now have been swept away by new work which studies quasar absorption lines at higher resolution than previously possible². Pettini *et al.* claim that the gas is largely neutral and exists in clouds some 10 million times smaller than previously assumed. Even by astronomical standards of imprecision, this is a dramatic alteration in our knowledge, and the paper has caused quite a stir. However, life is not simple for the bemused bystander trying to make sense of events: a competing group with data of similar quality do not reproduce these conclusions³. How can this be possible?

The region of the spectrum under dispute is the 'Lyman- α forest', which consists of typically 100 absorption lines per quasar of the $1,216\text{-}\text{\AA}$ $2p \rightarrow 1s$ atomic hydrogen transition. There is no dispute as to the nature of the lines (or at least most of them — see below), as they appear suddenly in the spectrum at wavelengths just shorter than Lyman- α emission from the quasar itself (the variation being due to the cosmological redshift of the absorbers). The lines are very narrow (equivalent to a spread of velocities of around 10 km s^{-1}), and there seems little doubt that we are detecting absorption of the quasar ultraviolet continuum by intervening material. But we have a rather poor set of data from which to learn about the absorbers: for each line, we deduce only the velocity width (for reasons unclear to me, the practitioners in this field choose to use a velocity parameter b which is $\sqrt{2}$ times the usual velocity dispersion) and the column density (N_c measures the integral of atomic number density along the line of sight through the cloud). The quasar continuum source is almost certainly much less than 1 parsec ($3 \times 10^{16}\text{ m}$) in size, so we learn only about a skewer through the absorbers that is absurdly small by comparison with galactic dimensions.

A most important result was therefore obtained when a close pair of quasars were found to have some absorbers in common⁴, implying that the clouds had

transverse sizes around the separation of the two sight-lines, at least 10 kpc. Armed with this size, a standard model could now evolve, because a size and a column density gives us a density. This is the density of only the neutral hydrogen of course, but the total density can be obtained because the gas will be photoionized by the quasars whose spectra it is busily absorbing. The

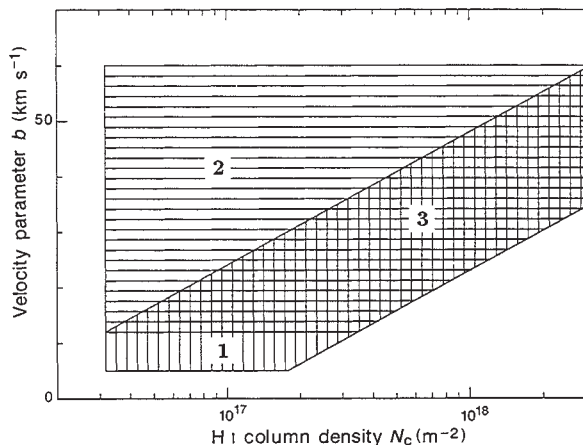


Fig. 1 A schematic diagram of velocity parameter against column density. Pettini *et al.* find weak lines that are very narrow (region 1) but none that are broad (region 2); Carswell *et al.* find the opposite. Both groups find lines in region 3.

density of ionizing photons from quasars at redshift 2.5 (typical for this work) is around $N_c = 0.5\text{ m}^{-2}$. A useful rule of thumb in this area is that the ratio of ionized to neutral hydrogen atoms scales as the ratio of the densities of ionizing photons and electrons, with 50 per cent ionization occurring when $N_c \approx 10^{15.2}\text{ m}^{-2}$. So, for a 'typical' cloud with column density $N_c = 10^{18}\text{ m}^{-2}$, a depth of 10 kpc implies a proton density of $5 \times 10^3\text{ m}^{-3}$ and a neutral fraction of 2×10^{-7} ; thus was born the picture of large, tenuous, highly ionized Lyman- α clouds.

An important consequence of the photoionization picture is that the gas must be hot: the typical energy per photoelectron is around 5 eV, which produces a temperature of about $10^{4.3}\text{ K}$ when shared with the nuclei. Radiative losses cool the gas rapidly if the temperature rises much above this level, so high ionization inevitably implies a gas temperature of this order. A temperature of 20,000 K corresponds to a velocity width of $b = 18\text{ km s}^{-1}$, which seemed to fit very nicely with the typical b values in the range $20\text{--}60\text{ km s}^{-1}$.

But all this has been challenged by Pettini *et al.*, who claim to detect many narrower lines, with b as low as 5 km s^{-1} . The temperatures implied by these lines are less than about 10^4 K and cannot be reconciled with photoionization. One is then forced to the opposite extreme of assuming that

the gas is largely neutral (which had been previously proposed on other grounds⁵). The photoionization formula now tells us that we need a neutral hydrogen density above $8,000\text{ m}^{-3}$, implying a size of only $4 \times 10^{-3}\text{ pc}$ for our 10^{18} m^{-2} cloud, where previously we had 10 kpc.

The necessity for these radical revisions to our ideas has been questioned by Carswell *et al.* on the basis of data of similar quality on a different quasar (Fig. 1): Pettini *et al.* find a tight correlation between N_c and b , which leads to very low b values for the systems of lowest column density, whereas Carswell *et al.* find there is a lower limit to b and many systems above the Pettini *et al.* correlation. The reason for these differences seems to come down to their methods of data reduction.

Carswell *et al.* use an automatic procedure which attempts to describe the whole spectrum by the minimum number of components necessary for a satisfactory fit within the noise of the data. It is clear that this can go wrong on occasion and fit a close blend of lines by a single over-wide component (Fig. 2), leading to the spurious occupation of area 2 in Fig. 1. Pettini *et al.* prefer the

more subjective approach of selecting by eye lines that were judged to be single isolated systems. The worry here is that there may be a tendency to pick out cases where noise fluctuations have produced a profile which is too narrow (see Fig. 2 again).

In a sense, it is hardly surprising that such problems arise: the signal-to-noise ratio of the data being used is in single figures, so it is hard to get very precise estimates of the cloud properties or to detect whether a given line is a blend. This is a source of hope: it should not be too hard to obtain data with noise around a factor of two smaller, in which case one would expect to be able to settle the current disputes quite clearly. At present, the situation is confused: although some of the lines in regions 1 and 2 of Fig. 1 may be spurious, it is hard to believe that they can all be. In the absence of new information, the best hope for the interested outsider would be if the groups concerned could collaborate to the extent of exchanging their existing data.

Even if the very narrow lines suggested by Pettini *et al.* are confirmed, it may still be possible to find an explanation within the old consensus about the Lyman- α clouds, because at least some of the lines in the forest could arise from transitions other than the hydrogen Lyman- α . At a given temperature, the velocity width of ions other than hydrogen ('metals' in

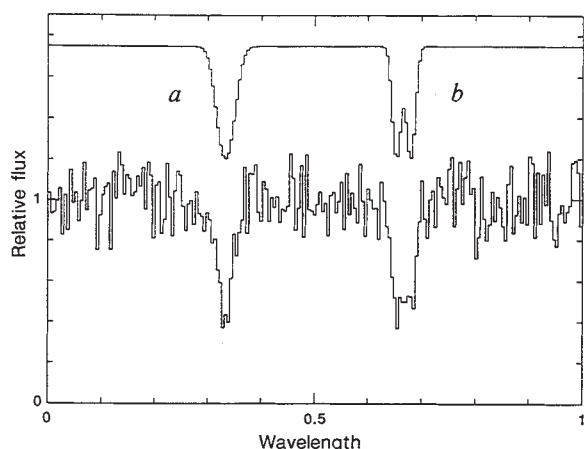


Fig. 2 Two simulated absorption lines made by adding gaussian noise (with signal-to-noise, $S/N = 8$) to *a*, a single line and, *b*, a close pair. The input profiles are shown offset for clarity. Noise has made line *a* too narrow; line *b* appears unresolved and hence will appear as a single line of spuriously large width.

astronomical jargon) is reduced by a factor $\sqrt{m_{\text{ion}}/m_{\text{H}}}$, where m is mass, leading to very narrow lines. Metal absorption systems in the Lyman- α forest are recognized by looking for absorption by other transitions of the metal ions at longer wavelengths. Pettini *et al.* remove 22 out of 101 lines in the Lyman- α forest region in this way. They have a further 24 lines with $b < 18$, and it is possible that at least some of these lines could be unrecognized metal systems.

However this controversy resolves itself, the old picture of very extended Lyman- α clouds may well be running into difficulties. New data reveal systems of lower and lower column density, with no evidence of any turnover in numbers

down to $N_{\text{c}} = 3 \times 10^{14} \text{ m}^{-2}$. We can turn these column densities into a size if we know a density, which may be provided by the theory of primordial nucleosynthesis⁶. This tells us that, at redshift 2.5, the mean proton density in the Universe was about 7 m^{-3} ; to have collapsed from the expanding background, one would expect a cloud to have a density at least a factor 100 higher than this. Photoionization then requires a neutral hydrogen density of more than 6 m^{-3} , leading to a depth of less than 0.2 pc for the lowest- N_{c} clouds. Thus, independently of the velocity widths, it seems very likely that the Lyman- α clouds are of sub-galactic dimensions.

That correlated absorption on scales of 10 kpc is seen may tell us that the clouds inhabit rather lumpy (proto) galactic haloes. As the data improve, we may hope to see this sort of idea put to the test relatively soon. □

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Deciphering the colour code

Anya Hurlbert

WE human beings can discriminate between a multitude of colours, giving them names such as lavender or chartreuse. Yet long before the stage where perception meets language, the brain must establish a more basic code for representing colours. The precise nature of that code is still unknown, despite an extensive history of psychophysical and electrophysiological studies. The results reported on page 235 of this issue¹ shed new light on the brain's colour code.

Trichromatic primates are able to see colour because light at different wavelengths excites the eye's three different cone types in different amounts (the L, M and S cones, also called R, G and B, are maximally stimulated by long-, middle- and short-wavelength light respectively). Thus colours are first encoded by triplets of cone responses in the retina. Yet this code specifies colours neither uniquely

nor efficiently. The colours we see at one place in an image depend not only on the cone responses there but also on surrounding cone triplets. The colours we see at one time also depend on those we saw moments before: the cones continuously adapt to changes in the prevailing light intensity and spectral composition, shifting both their sensitivity and setpoint of operation. The cone-response code is not efficient because the range of wavelengths to which the distinct cone types respond overlap greatly. The responses of the L and M cones especially are highly correlated: if the L cone is active, the M cone is likely to be also.

So the brain has evolved second-stage mechanisms that transform the cone triplets into a more reliable and discriminatory code. In the three-dimensional space spanned by all possible cone triplets, this transformation amounts to a rotation of

the coordinate axes defined by the cone sensitivities. In theory, any new set of three mutually orthogonal axes can be used to recode the cone triplets. In practice, the brain appears to choose axes that best discount redundancies and enhance differences in the cone responses². It does so by pitting the cone responses against each other. Few colour scientists would disagree that this colour-opponent processing occurs, yet few would agree exactly how.

The disagreement turns on exactly what direction the opponent-colour axes take in colour space. Traditionally, the directions have been defined by the locations of complementary pairs of unique hues: one axis links unique blue and yellow; another, unique red and green. The third axis is a luminance axis which links white and black. The sensitivities of these opponent-colour mechanisms have been defined by hue-cancellation experiments³.

Another method of defining the opponent-colour directions has yielded different results. Krauskopf, Williams and Heeley⁴ adapted observers to fields of colour that varied sinusoidally in time, then measured their sensitivity to brief flashes of coloured light. Because cone adaptation is rapid, and the mean chromaticity and luminance of the adapting field were kept constant, the cone sensitivities themselves were not altered. When the adapting fields were varied in colour along a 'cardinal' direction, detection thresholds were most elevated for test stimuli along the same direction, and unchanged for tests along orthogonal directions. When the adapting direction was intermediate between any two cardinal directions, the detection thresholds for all test stimuli were uniformly elevated.

One cardinal axis is aligned with the unique red-green axis, labelled L-M because it receives inputs only from the L and M cones in opposition⁵. But the other axis deviates significantly from the unique blue-yellow direction and instead lies close to the tritanopic confusion line, along which the ratio of L and M cone activities remains constant and only the S cone activity changes. This axis is labelled S, or more accurately, S-(L+M). The third axis, the luminance mechanism, responds to the sum of the L and M cone activities.

Other psychophysical results support the idea that, beyond the retina, colours are encoded by the relative excitation of these three mechanisms (see, for example, ref. 6). Most recently, Krauskopf and Farell⁷ have reported that the colour information used in motion perception appears to be analysed along the cardinal directions. Two independently moving grating patterns join in coherent motion when each is formed by a variation in colour along the same cardinal direction. If the colour variations are along

Hunting the helix

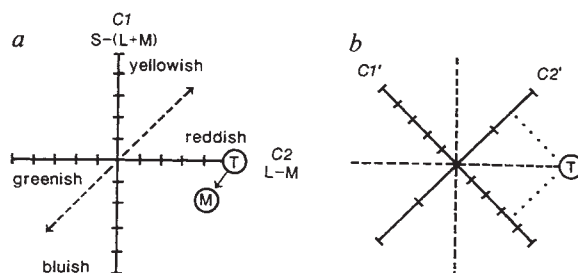
How does a DNA-binding protein recognize its allotted site? If it can 'feel' inside the helical grooves it will encounter the edges of the bases; but the helix geometry may itself vary with sequence. G. G. Privé *et al.* (*J. molec. Biol.* **217**, 177–199; 1991) compare the structures of three crystallographically isomorphous decanucleotides in the form of self-complementary B-DNAs. The width of the minor groove and other features (including hydration pattern) vary markedly with composition. Crystal packing perturbs the structure at some sequence conjunctions, which therefore define the intrinsically 'soft', distortion-prone parts of a helix. In a following report by the same group, K. Yanagi *et al.* (201–214) draw together the 11 available high-resolution structures and look for empirical rules. A general predictive algorithm is not yet within reach, as the structure depends not only on nearest neighbours but also on the base pairs on either side: of the 136 four-base-pair combinations only 33 are represented in the known structures.

Gut reactions

SMOKERS who are also drinkers will find cause for rumination in a report from an Australian group published in the *British Medical Journal* (**302**, 20–23; 1991) — as will traffic police. R. D. Johnson and colleagues find that cigarette smoking delays the absorption of alcohol into the bloodstream. This is a result of the slowing of passage of the stomach contents into the small intestine, the principal site of alcohol absorption. The authors point out that the finding has implications for legal proceedings in suspected cases of drunken driving, because the back-calculation of blood alcohol levels to the time of a vehicle accident is more complicated than it might have seemed.

Bright ideas

A TEAM at the Technische Hochschule Darmstadt has devised a bright X-ray source, tunable from 5 to 20 keV, using the remarkable phenomenon of channelling radiation. The physical principle exploited by H. Genz *et al.* (*Appl. Phys. Lett.* **57**, 2956–2958; 1990) is the radiation of photons by high-energy electrons passing along a chain of atoms in a perfect crystal: varying the electron energy tunes the X-rays. Although the X-ray intensities obtained so far are lower than those from conventional synchrotron sources, the new device relies only on an electron accelerator a few metres long (and a small crystal), not the vast storage rings used at the dedicated laboratories such as at Daresbury in the UK and Grenoble in France. As a result, similar systems could easily be deployed in university or hospital laboratories.



a, A constant-luminance plane in colour space ($L+M = \text{constant}$). The axes $C1$ and $C2$ are aligned along the cardinal axes: along $C1$ ($S-(L+M)$), colours vary from greenish-yellow to reddish-blue; along $C2$ ($L-M$), colours vary from reddish to greenish. After an observer adapts to a field varying between orange and turquoise along the dotted line, the hue of a test light on axis $C2$ (T) is shifted in the bluish direction (M). b, This could result from a realignment of the axes in the adapted field. In the new coordinate system, the test light stimulates both $C1'$ and $C2'$, and the mechanism underlying $C2'$ is desensitized. To match T, M must therefore have components along both $C1$ and $C2$. (The predicted hue shift is larger than observed: It may be that the axes are realigned all across the visual field, but the sensitivity of $C2'$ is diminished only in the adapting field.)

orthogonal directions in colour space (for example red–green and purple–yellow) the patterns do not cohere but slip against each other. If the colour variations are along intermediate directions, they again cohere. It seems that colours are analysed into cardinal components before they contribute to motion perception.

But might there be yet a third stage of colour encoding? If the perception of chartreuse is represented at the second stage by a combination of activities in the $L-M$ and $S-(L+M)$ opponent mechanisms, then there could be a higher-order mechanism that lights up when it registers that combination. If so, there should be many such mechanisms, and the colour code at that level would consist of a large array of their activities.

Using a technique similar to that of Krauskopf *et al.*¹, Webster and Mollon find surprisingly different results. These imply that such a third stage does exist. Instead of measuring the detectability of faint test flashes, Webster and Mollon asked observers to match the colour of supra-threshold test lights after adapting to temporally modulating fields. As one would predict from the results of Krauskopf *et al.*, when the observers adapted to colour variations along a cardinal direction, the greatest change in colour appearance occurred for test lights along that direction and the least change for test lights along the orthogonal direction. But for some observers, this was also true for intermediate adapting directions: for every adapting direction tried, the greatest change was in that direction, the least in the orthogonal direction. Krauskopf *et al.* found hints of a similar effect in their original study defining the cardinal directions: for some observers, some selective desensitization also occurred for oblique directions. Thus there seem to be

many mechanisms involved in encoding colour, each tuned to a distinct direction in colour space and each independently susceptible to desensitization.

This technique relies on the time-honoured tenet of psychophysics, that adaptation reveals selective mechanisms by fatiguing them. But many psychophysicists are themselves weary of fatigue as the explanation for adaptation. Barlow and Foldiak⁸ argue that the term fatigue obscures the natural purpose of desensitization: the visual system may deliberately reduce its sensitivity to frequently occurring combinations or 'contingencies' as a way of storing information about the natural world. This enables it to be

more sensitive to the unexpected.

What contingencies might be stored for colour? One possibility is suggested by Lee's observation⁹ that the locus of daylight chromaticities forms a straight line in the constant-intensity plane of cone responses, and that this line falls close to the blue–yellow axis. So the visual system seems to have adapted to the natural variations in light it encounters by aligning one of its colour-coding axes along the same direction, and the others in the orthogonal directions.

This argument leads to another interpretation of Webster and Mollon's results. First note that they are not exactly what one would expect from truly independent mechanisms. If each discriminable direction in colour space were represented by a strictly independent mechanism, then variations along one direction would not affect the activity of mechanisms tuned to other directions. Test stimuli placed anywhere except on the adapting axis should therefore appear unchanged, not just those placed on the orthogonal axis.

The results are perhaps more consistent

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with adaptation causing a rotation as well as desensitization of a set of orthogonal axes, which in their natural state are aligned along the cardinal directions. Exposing the visual system to modulation along an oblique direction might cause the original axes to realign along that direction, and those orthogonal to it, to match the new environment (see figure). At the same time, sensitivity to changes along the adapting direction are reduced: the visual system attenuates expected changes and amplifies unexpected ones. Following this argument, one might predict different results for the interaction of colour and motion when observers first adapt to fields modulated along oblique directions in colour space. Patterns formed by variations along two orthogonal cardinal directions might then cohere in motion.

Investigating the sensitivities of cell

populations in the visual system should provide essential clues to the brain's colour code. Already it has been shown that in the parvocellular layers of the lateral geniculate nucleus, cell sensitivities cluster along the two cardinal directions¹⁰. In layers II and III of striate cortex, blue-yellow cells are segregated from red-green cells in metabolically active 'blobs'¹¹. Other results suggest that cells in higher visual cortical areas may be broadly tuned to several directions in colour space¹². Yet the physiological data are still incomplete, and these psychophysical results emphasize that care must be taken not to perturb the colour code while deciphering it. □

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Edinburgh). In monkeys, changes in the antigenic profile of a cloned line of *P. knowlesi* can be caused by either a point mutation or a partial deletion of the gene for the antigen involved (Wellems). Taken together, these observations demonstrate the importance of allelic diversity, mutation and changes in gene expression in parasite variation. In the rodent malaria parasite *P. yoelii* the situation might be even more complicated, and data were presented at the meeting showing that several similar, but not identical, copies of a gene coding for a protective antigen can exist in the genome, suggesting that multigene families might be involved in the expression of variant antigens (A. A. Holder, NIMR, Mill Hill).

Maintenance

There are differing ideas as to how variant characters arise and are maintained in parasite populations — some favour random drift, others the more conventional view of selection pressure by, for example, the immune response. Given the extent of variation already recognized, there is room for both views. But one consequence is that it will be impossible to take a synoptic view of variation when designing drugs or vaccines, so for the time being it is necessary to consider a pragmatic approach. Studies on variation have also produced evidence that there are non-variable sequences for some antigens, and S. Herrera (Universidad del Valle, Colombia) discussed the possibility of using such conserved regions in 'cocktail' vaccines. And in contrast to the widely held view that all the stages in the malaria life-cycle are antigenically different, certain antigens are present in more than one stage — these might be components of future vaccines (B. Sina, Biomedical Research Institute, Rockville).

The problems associated with the lack of new drugs were also covered (P. I. Trigg, WHO, Geneva; N. J. White, University of Oxford). Encouraging results have been reported for halofantrine and artemisinin derivatives, drugs that have not yet been released, but there are already reports of resistance to the most recently introduced drug, mefloquine. Given the ability of the malaria parasites to produce mutations, and to express or delete certain proteins, the development of new drugs or rational vaccines is going to be a lengthy task. □

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MALARIA

Variation and vaccination

F. E. G. Cox

FOLLOWING closely on the debate¹⁻⁴ about the 'clonal' nature of human parasitic protozoa, the point at issue being the extent of their genetic diversity, a recent meeting* provided the chance to take stock of all aspects of variation in one of the most important groups — that whose members cause malaria.

The genetic diversity of malaria parasites, particularly of the malignant tertian form, *Plasmodium falciparum*, can now be analysed in great detail by molecular, biochemical and immunological techniques. From such studies, it is clear that considerable variations occur both within and between different populations, and between parasites in a single infection, and that this variation is extremely important in both drug therapy and in susceptibility to immune attack. Genetic complexity has several components: (1) allelic variation of many genes, as exemplified by the merozoite surface antigen MSA-1; (2) mutation, as exemplified by widespread resistance to drugs; (3) variation in expression of different genes, as seen in many stage-specific antigens; and (4) the occasional absence of genes due to chromosome deletion events. Added to this is recombination during meiosis in the mosquito vector, which results in the production of parasites with novel combinations of genes.

The two drugs most widely used against malaria, particularly for prophylaxis, are chloroquine and the anti-folate pyrimethamine. Resistance to them takes different forms (A. Cowman, Walter and Eliza Hall Institute of Medical Research,

Melbourne). Pyrimethamine resistance, which has multifocal origins, can be explained in terms of point mutations in the gene for dihydrofolate reductase; by contrast, resistance to chloroquine, which seems to have spread from a few foci, is thought to have a multigenic basis, although there is evidence that only a single gene is involved in a cloned line from South East Asia (T. E. Wellems, NIH, Bethesda). In Thailand (S. Thitong, Chulalongkorn University, Bangkok) many isolates are resistant to both drugs, and clones derived from such isolates vary in their susceptibility to them. Hybridization between such drug-resistant parasites in mosquitoes generates parasites with different resistance phenotypes and can give rise to multi-drug-resistant forms.

Diversity

Molecular techniques can also be used to identify polymorphisms in parasite proteins, particularly the surface antigens recognized by the immune system. The extent of this diversity is astonishing — in one Sudanese village each of 29 isolates, collected within a few days of each other, contained different genotypes (D. Walliker, University of Edinburgh); and in Papua New Guinea four different kinds of the *P. falciparum* S-antigen have been identified in a single household (R. F. Anders, Walter and Eliza Hall Institute of Medical Research, Melbourne). Similar studies have shown that in Africa and South America most infections are mixed, but in Sri Lanka most *P. vivax* infections were found to consist of single clones possibly correlated with low intensity of transmission (R. Carter, University of

*Symposium on Genetic Variation of Malaria Parasites Leiden, 14–16 November 1990. Proceedings to be published in *Acta Leidensia*.

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In the eye of the beholder

C. Martin Gaskell

How similar are the different types of quasar activity? How far can they be unified? To what extent does our classification of an active galaxy merely depend on the angle at which we view it? These were some of the main questions addressed at a recent workshop, *Grand Unified Theories of Active Galactic Nuclei**, the word "theories" being carefully chosen because three main schemes were discussed.

The first, originally proposed by Blandford and Rees¹, is that so-called BL Lac objects (highly variable and highly polarized objects which lack obvious strong emission lines) are really low-power radio galaxies (of so-called Fanaroff–Riley class I, or FR I sources) whose relativistic radio jets are aimed at us like a searchlight. This unification scheme is now generally accepted, and is a necessary consequence of special relativity. Many extragalactic radio sources show apparent superluminal (faster than light) motion, which must be due to relativistic motion close to the line of sight. Relativistic motion produces very anisotropic emission ('relativistic beaming' along the direction of motion). Therefore, when the beam is aimed at us, its radiation dominates what we see. When it is not, the object seems much fainter.

Centaurus A

Blandford and Rees's explanation has now been shown to work well, quantitatively. Both the extended radio emission and the extended optical emission-line properties of BL Lacs match those of low-luminosity FR I radio galaxies. And the observed relative numbers of BL Lacs to FR I radio galaxies is that expected from the model. Interesting new evidence supporting the model is that the energetics of the emission-line gas in the direction of the radio jets in the nearby FR I radio galaxy Centaurus A require an X-ray flux about 200 times stronger than seen from the Earth (R. Fosbury, Space Telescope Science Institute). The flux the gas clouds see is in fact comparable to that of the prototypical BL Lac object, BL Lac, itself. If the Earth were in a different place, we might classify Cen A as a BL Lac object.

The relativistic beaming invoked in Blandford and Rees's model for BL Lacs is a case of intrinsic beaming, a property of the galaxy's central engine itself. In the second scheme, an attempt to unify Seyfert 1 and 2 galaxies, extrinsic beaming is required. Seyfert 1 galaxies show broad emission lines (due to photoionized gas with a density of 10^{10} cm^{-3} moving at speeds

of up to a few per cent of the speed of light) whereas Seyfert 2s show only narrow emission lines (from slower-moving, lower-density gas). Keel² discovered that Seyfert 1 galaxies are preferentially orientated pole-on towards us (Seyfert 2s are not), but the idea that perhaps all Seyfert 2s are Seyfert 1s seen from the side, with dust obscuring the broad line region, was at first not generally accepted. Seyfert 2s are not strong sources of X-rays, unlike Seyfert 1s, and their radio structures were thought to be different. Also, the broad lines in a number of Seyfert 1s can disappear almost completely at times, making them look like Seyfert 2s.

The Seyfert unification scheme was strengthened, however, by the discovery³ of the spectrum of a Seyfert 1 galaxy in the polarized light of the classic Seyfert 2 galaxy NGC1068. Apparently in NGC 1068 there is a physically thick nuclear torus blocking our direct view of this galaxy's nucleus, which we can detect only through light scattered off electrons above the disk or reflected off nearby dust clouds. That is, the thick torus causes extrinsic beaming.

Further polarization observations⁴ have since revealed other Seyfert 2s with hidden broad lines, strengthening the case for unification. Furthermore, some of the earlier objections to the scheme have been discounted: new radio observations⁵ have largely eliminated the systematic differences thought to exist between the galaxies' radio properties; and the X-ray spectra Seyfert 2s now turn out to be similar to those of Seyfert 1s. Where differences remain (as in the properties of narrow emission lines), the debate at the workshop centred on whether or not they can be explained in a unified theory by orientation effects and what the role of selection effects is in different samples.

Perhaps the most interesting new evidence to support extrinsic beaming was the observation⁶ of gas in and around the host galaxies, showing that the nucleus lights these up only in cones aligned along the radio axis (see figure). The exact nature of the torus and its effect at various wavelengths have yet to be determined. S. Simkin (Michigan State University) presented evidence that the morphology of the spiral arms of the host galaxies differ between Seyfert 1s and 2s. If this cannot be explained away as some selection effect it supports the idea that there are differences between Seyfert 1 and 2 galaxies more fundamental than simple orientation.

The third unification scheme considered at the workshop is an extension of a proposal of Orr and Browne⁷. This is

that there is a progression among the stronger extended radio sources (the so-called FR II sources): at one extreme, there are optically weak objects showing only narrow emission lines from low density gas (so-called narrow-line radio galaxies); then radio galaxies showing broad emission lines, and the optically brighter quasi-stellar objects (QSOs) with lobe-dominated radio emission; and finally QSOs with core-dominated radio emission. It is postulated that that sequence is explicable if the first extreme is an FR II source seen sideways on and the others have the radio jet progressively turned towards us. This scheme is a combination of the previous ones and invokes both intrinsic and extrinsic beaming. Although extensive evidence including new polarization observations (B. J. Wills, University of Texas) supports it, as with the Seyfert unification scheme, a few remaining problems raise doubts as to whether this is the whole story.

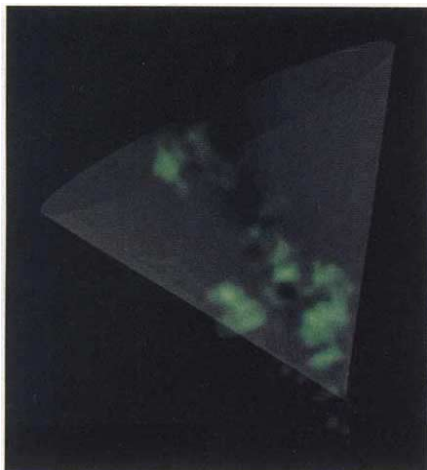
Bimodality

What differences cannot be explained by current unified theories? The main one everyone seemed to agree on is that between radio-loud and radio-quiet quasars. The most recent observations confirm that there is indeed a fundamental bimodality in the radio power of quasars. Also the difference between FR I and FR II radio sources cannot be explained in terms of orientation, and broad-absorption-line quasars refuse to fit into the schemes.

Of the other parameters needed in the unified quasar theories, the most obvious are the mass of the central black hole and, perhaps, the rate at which matter is accreted onto it. This accretion mechanism is generally accepted as being the basic source of luminosity in active galactic nuclei. S. Phinney (California Institute of Technology) noted that the most important relation for observers to produce is that between luminosity and mass. Progress in learning about the size, structure and kinematics of the broad-line region from variability studies is now making it possible to verify previous indirect estimates of the mass–luminosity relationship for quasars⁸; the results are consistent with accretion occurring at a relatively modest rate, a few per cent of the so-called Eddington rate (the maximum possible

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The core of the galaxy NGC1068 as recorded recently by the Faint Object Spectrograph on the Hubble Space Telescope. The image clearly shows small clouds of ionized gas illuminated by the conical extrinsic emission from the galaxy's nucleus. (The extent of the cone, aligned with the radio axis, is indicated by the artificial computer-generated graphic.)

rate for steady spherically symmetric accretion) over a range of black hole masses. Another factor to include is the quasar's environment: rather surprisingly, no one at the workshop mentioned the possible role of galaxy mergers.

SOLID-STATE PHYSICS

Single-molecule probes

J. L. Skinner

UNDERSTANDING the nature of molecular dynamics in condensed phases has been a central theme in physics and chemistry for many years. On page 225 of this issue¹, Ambrose and Moerner report monitoring the fluorescence of single pentacene molecules in a molecular crystal of *p*-terphenyl. The fluorescence frequency of the pentacene molecule can jump by small amounts, the mean time between these jumps being 20–420 s. The authors interpret these frequency jumps as the result of changes in the local molecular environment of the pentacene molecule. Thus this experiment provides the first direct measurement of these local motions, and paves the way for detailed analyses of molecular dynamics in condensed phases.

The use of probe molecules that can be monitored spectroscopically is a time-honoured method for determining local structure and dynamics in condensed phases. One hopes, of course, that the probe molecule will not perturb the structure and dynamics of the host system to any significant extent. What one learns from the optical spectroscopy of dilute impurity probes in crystals and amorphous solids depends on the timescales involved. For times shorter than the

What are the biggest remaining puzzles? Very fast X-ray variability is a severe difficulty for any model. About a third of the Seyferts looked at by EXOSAT vary on a timescale of hours; five varied significantly in only 20 minutes. Some ultrafast radio variability also seems to be intrinsic to the sources. The origin of the jets remains a problem. Accretion disks are frequently suggested to be the means of extracting the gravitational energy to power quasars (and are also a means of providing some of the collimation for beaming), but they present serious observational and theoretical problems. One is the lack of hydrogen bound-free edges in the spectra in either emission or absorption²: such edges are seen in the accretion disks that surround cataclysmic variable stars but not quasars. Other problems include the relatively low polarization of quasars and the uniformity of apparent temperature of the ultraviolet spectrum in different objects and in individual objects as they vary. So although the unified theories have come a long way, we are still far from having a single grand unified theory of quasars. □

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this process over all impurities is known as spectral diffusion, which is thought to account for the discrepancy between photon-echo dephasing times and hole widths from hole-burning experiments for resorufin in organic glasses³ — the former experiments measure only short-time dynamics, whereas the hole widths also reflect a contribution from the longer-timescale spectral diffusion.

The direct observation of molecular dynamics from impurity spectroscopy¹ relies on the detection of single molecules, which has recently been achieved through double-modulation absorption⁷ and fluorescence⁸ techniques. The key to the detection of single molecules in crystals involves both the spatial and the spectral isolation of the impurities, achieved by using very thin crystals and small laser spots and by tuning the laser frequency significantly away from the central maximum of the absorption lineshape. The fluorescence technique allows one to see the frequency jumps of individual probe molecules.

These can provide much more information than the average spectral changes measured in more traditional experiments, such as hole burning, that involve many impurities. Thus one could measure distributions of jump times and frequency changes and the correlations between them, or, for example, the frequency-frequency time-correlation function for a single molecule. The temperature dependence of these quantities should enable one to determine, by comparing with theoretical models, the specific molecular motions of the host that are responsible for the frequency jumps.

One possible limitation of the technique is that only those impurity molecules way out in the wings of the inhomogeneous distribution, which by definition means those molecules in atypical environments, can be spectrally isolated. One would, of course, like to develop a technique that could measure the environmental changes in real time near typical impurities. Nonetheless, the detailed data that are sure to follow from the present generation of experiments will provide grist for the mill for quite some time. □

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fluorescence lifetime of the impurity, small-amplitude thermal motions of the host molecules occur that are statistically the same for each impurity. These motions rapidly vary the resonance frequency of the impurity, and thus produce what is known as homogeneous line broadening of the absorption or fluorescence spectrum^{2,3}. On a timescale somewhat longer than the excited state lifetime, each impurity sees an average environment that is time independent and is different from the other impurities owing to disorder in the host crystal or glass. This produces inhomogeneous broadening. The actual absorption or fluorescence spectrum at low temperatures (1.5–10 K) is usually predominantly inhomogeneous. Nonetheless, various techniques — fluorescence line narrowing⁴, hole burning⁵, photon echo⁶ and statistical fine structure⁵ — have been developed that allow a determination of the homogeneous linewidth in these inhomogeneously broadened systems.

On a timescale substantially longer than the excited state lifetime, the time-averaged host environment of any particular impurity may change significantly, leading to a change in the absorption or fluorescence frequency. The average of

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Sticky sugars for selectins

Timothy A. Springer and Laurence A. Lasky

CELL-adhesion research has suddenly become sweeter. Only a year after three members of a family of cell-adhesion molecules were found to have lectin-like N-terminal domains^{1,2}, a spate of reports has appeared identifying the sticky sugar structures that are their putative ligands (see figure). That a good portion of these papers come from biotechnology companies reflects the fact that carbohydrate cell-adhesion ligands are relatively small, simple structures; thus the synthesis of drug analogues that will be tested for anti-inflammatory and anti-thrombogenic properties cannot be far off.

The role of carbohydrates in cell interactions started to receive close attention with the recognition that proteoglycans and glycosaminoglycans are important components of the extracellular matrix. Heparan, chondroitin sulphate and hyaluronic acid bind to many proteins in the matrix. In theory, carbohydrates are well suited for specific recognition — monosaccharides have many hydroxyls that can be linked *O*-glycosidically in both branched and linear arrays, so far more carbohydrate than protein structures can be created from the same number of monomers. Protein-protein recognition has received most of the limelight in studies of cell-cell adhesion, because this is the dominant and perhaps exclusive form of recognition by adhesion receptors of the integrin and immunoglobulin super-families¹. But work on carbohydrates has advanced rapidly with the determination of many complex structures by fast-atom-bombardment mass spectrometry, and of cell type-specific carbohydrate structures and linkages that are regulated during cell differentiation by selective expression of glycosyl transferases. Recent examples of the identification of carbohydrate receptors on cell surfaces include one on sperm which recognizes an *O*-linked carbohydrate in the zona pellucida³; and CD44, a widely distributed surface receptor for hyaluronic acid^{4,5}.

Now a family of adhesion-receptor glycoproteins critical to interactions of circulating cells within the vasculature must be added to the list. This family has been designated the selectins (ref. 6) or LEC-CAMS (ref. 2). The name selectin capitalizes on the derivation of 'lectin' and 'select' from the same Latin root, meaning to separate by picking out, and is in keeping with those of the immunoglobulin, integrin and cadherin adhesion-receptor families. The homing receptor selectin, also called gp90^{nc1}, LAM-1, LEC-CAM-1 or LECAM-1, is expressed on leukocytes and facilitates their binding to endothelium during lymphocyte recirculation

through peripheral lymph nodes and neutrophil emigration at inflammatory sites. The PADGEM, GMP-140 or CD62 molecule is a granule-associated glycoprotein of platelets and endothelial cells that is brought to the cell surface after stimulation by thrombogenic agents, allowing these cells to bind neutrophils and monocytes at the site of tissue injury. The ELAM-1 glycoprotein is synthesized by endothelial cells in response to inflammatory agents and promotes adhesion of neutrophils, monocytes and a subpopulation of lymphocytes. These proteins thus mediate adhesion of leukocytes to endothelium and platelets during inflammation and clotting^{1,2,7}.

Clues that the selectins are carbohydrate-specific came from two directions. Early studies had shown that the homing receptor selectin could bind to algal or yeast polysaccharides rich in fucose sulphate or mannose phosphate; that these and related monosaccharides could competitively inhibit binding to lymph-node endothelial cells; that binding is Ca²⁺-dependent; and that binding is abrogated by neuraminidase treatment of the endothelial cells⁷. Subsequent cloning of complementary DNAs revealed that the homing receptor, ELAM-1, and CD62

are members of a structurally related adhesion-receptor family that has an N-terminal domain homologous to Ca²⁺-dependent (C-type) animal lectins (see figure). Some animal lectins are found at cell surfaces, and include hepatic receptors that are specific for terminal galactose and fucose⁸. Although the homing receptor was the first selectin thought to have a carbohydrate ligand⁷, the identity of the biological ligand remains unclear; the recent results concern the ligands of the other two selectins, CD62 and ELAM-1.

The CD62 ligand, known to be present on neutrophils and monocytes, was putatively identified by screening for monoclonal antibodies that would block binding of these cells to CD62. CD15, also known as Lewis x (see figure), was thus identified as a component of the ligand, and this was supported by the ability of a purified Lewis x adduct of lactose found in human milk to partially inhibit adhesion at relatively high concentrations⁹.

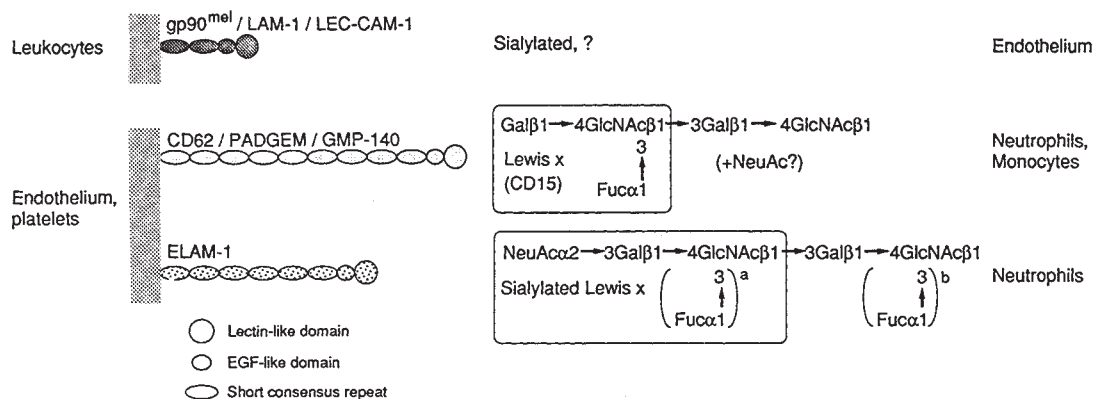
No less than five independent papers have appeared within the past three months on the structure of the ELAM-1 ligand and the enzymes that synthesize it. Neutrophils are rich in Lewis x and sialylated Lewis x (refs 10 and 11; see figure). These are $\alpha(1\rightarrow3)$ fucosylated derivatives of polylactosamine that are found at the nonreducing termini of glycolipids, *O*-linked carbohydrates and *N*-linked oligosaccharides. Expression of sialylated Lewis x in neutrophils and a variety of cell

When beauty is only skin deep



THE appeal of this tempting punnet of fruits from the tropical tree *Elaeocarpus angustifolius* lies in iridescence, a property more usually associated with birds and butterflies than higher plants. Ripe *Elaeocarpus* fruits are iridescent owing to remarkable structures called iridosomes in their skins, described by D.W. Lee on page 260 of this issue. Thin-film interference in stacks of polysaccharide fibrils — the right thickness (78 nm) to reflect blue light with a wavelength of 439 nm — allows the fruits to be brightly coloured and yet still admit the yellow-green light needed for photosynthesis. So not only do the fruits stand out in the forest gloom, they allow the trees to soak up every drop of available light right up to the final stages of fruit maturation. Henry Gee

Selectins and their ligands. The selectins have N-terminal lectin-like domains, an epidermal-growth-factor-like domain, and varying numbers of short consensus repeats as found in proteins regulating complement activation^{1,2}. Fucoses in parentheses in the ELAM-1 ligand: fucosylation was reported at sites marked a (ref. 14), a or a and b (ref. 13), and b (ref. 10). NeuAc, sialic acid; Gal, galactose; GlcNAc, N-acetyl glucosamine; Fuc, fucose.



lines, including mutants with altered $\alpha(1\rightarrow3)$ fucosylation, correlates with ELAM-1-dependent adhesiveness¹²⁻¹⁴. Furthermore, monoclonal antibodies specific for sialyl Lewis x, as well as a difucosylated sialyl Lewis x glycolipid or sialyl Lewis x-containing mucin added as competitors, inhibit binding of neutrophils to endothelial cells or a purified ELAM-1 chimaeric globulin^{13,14}. Negative cells can be converted to ELAM-adhesive cells positive for sialyl Lewis x by transfection with an $\alpha(1\rightarrow3/1\rightarrow4)$ -fucosyl transferase that is the product of the Lewis blood group locus¹². A distinct fucosyl transferase, with a more restricted $\alpha(1\rightarrow3)$ specificity and an expression limited to myeloid cells that bind ELAM-1, was isolated by expression cloning, using a monoclonal antibody to an undefined carbohydrate structure that acts as an ELAM-1 ligand¹⁵. Transfection showed that adhesion of cells to ELAM-1 can be regulated by expression of this single sugar transferase.

In a daringly direct approach to ligand identification, a different group fractionated a glycolipid extract of neutrophils and assayed fractions for binding to ELAM-1-transfected cells¹⁶. Mass spectrometry of a highly purified fraction demonstrated a structure similar to sialyl Lewis x, except that fucose is in $\alpha(1\rightarrow3)$ linkage with the penultimate rather than the ultimate N-acetyl glucosamine (see figure). The sialylated, penultimately fucosylated structure is identical to CD65 (ref. 17), expression of which was shown by another group not to correlate with ELAM-1 binding¹⁴. But the difucosylated structure clearly binds¹³, and all groups agree that enzymatic removal of sialic acid or fucose ablates ligand activity. In summary, ELAM-1 recognizes a family of sialylated, fucosylated polylactosamines, and the critical determinant seems to be sialyl Lewis x.

Although these results forcefully and elegantly demonstrate the role of carbohydrates as specific mediators of cell adhesion, many issues remain to be resolved. The structures identified so far may represent only the minimum requirements for selectin binding; given the enormous diversity inherent in carbohydrates, alter-

native minimal structures, or more complex structures containing substituents that increase affinity, might be found. A carbohydrate structure containing sialic acid seems to have higher affinity than the unsialylated Lewis x structure for CD62, because neuraminidase treatment of neutrophils reduces CD62-mediated adhesion^{18,19}. Uncertainty about the exact relationship between Lewis x (CD15) and the CD62 ligand arises from the incomplete inhibition by antibodies to CD15 of binding to purified CD62 in the original report⁹, and a failure of a different monoclonal antibody to CD15 to inhibit this interaction in a subsequent report¹⁹.

That there coexist on the cell surface subpopulations of ligand structures that differ in affinity, or in accessibility owing to the nature of their linkage to the cell surface, is suggested by the observation that neutrophil activation decreases adhesiveness for ELAM-1 (ref. 20) without decreasing binding sites for sialyl Lewis x monoclonal antibody¹⁴. Ligand structures also seem to differ from cell to cell. The ELAM-1 ligand structures on natural killer cells and granulocytes are not the same, because although both cells express sialylated Lewis x (ref. 21) and bind to ELAM-1 (ref. 15), a monoclonal antibody that binds to a carbohydrate determinant on the ELAM-1 ligand on granulocytes does not bind to natural killer cells¹⁵. This antibody has a specificity distinct from monoclonal antibodies specific for sialylated Lewis x, and probably binds to a less terminal carbohydrate, because its determinant does not involve sialic acid. Still another ligand seems to exist on a subpopulation of skin-homing memory lymphocytes — these cells bind to ELAM-1-transfected cells^{22,23}, yet lymphocytes do not express sialylated Lewis x (ref. 21). The determinants of receptor specificity and affinity may be overlapping but distinct from determinants of antibody specificity, and both are likely to be structurally heterogeneous.

At their meetings, glycobiologists often give one another pep talks about the biological role of glycoconjugates. The many oligosaccharide structures attached to proteins or lipids that have been deter-

mined over the past decades, sometimes without a clue as to their functions, have led to a crowning achievement in the field of cell adhesion. Interesting refinements or variations in the elegant yet simple structures of the two reported selectin ligands, and identification of the third, will probably obsess researchers in this suddenly hectic field before they can turn to the design of synthetic analogues that could result in pills that are sugar-loaded as well as sugar-coated. Adhesion inhibitors are promising candidates for anti-inflammatory and anti-thrombogenic drugs. And because sialyl Lewis x is expressed on diverse tumour types²⁴, including colon carcinoma cells that bind to ELAM-1 (ref. 25), such drugs might also be anti-metastatic. □

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Ironing out greenhouse effects

Robin S. Keir

It has been proposed that CO₂ could be removed from the atmosphere and thereby its greenhouse effect reduced by using the so-called 'iron hypothesis' of Martin¹. According to this idea, iron is the limiting nutrient for biological growth in Antarctic surface waters, which have abundant phosphate, nitrate and silicate. By fertilizing this area with iron, phytoplankton growth would be greatly stimulated, and the surface-water's partial pressure of CO₂ would be greatly reduced — by more than 150 parts per million (p.p.m.). But even if the fertilization were completely successful, the effect on atmospheric CO₂ would be much smaller according to Peng and Broecker on page 227 of this issue². Their model calculations show that after 100 years of successful fertilization, the atmospheric CO₂ content would be reduced only by about 30 p.p.m., an amount which is also small in comparison to the hundreds of parts per million the concentration is projected to rise during this time owing to fossil-fuel burning.

The authors point out that the problem is that the capacity of the Antarctic surface water by itself to take up atmospheric CO₂ is very small. Therefore this region acts only as an interface through which additional CO₂ could be sequestered from the atmosphere and transferred into the large volume of intermediate and deep waters. The rate of this transfer depends upon the nature of the circulation: the speed with which the upper waters are replaced and the extent to which they remain in contact with the atmosphere afterwards.

The model simulations on page 228 (their Fig. 3) illustrate this aspect quite clearly. Initially there is a transitory period of a few years during which the CO₂ partial pressure (p_{CO_2}) in the surface water decreases rapidly and subsequently recovers to within 30 p.p.m. or so of the atmospheric value. The latter evidently occurs because of rapid exchange of CO₂ across the sea surface. After this transient, the surface p_{CO_2} maintains a nearly constant disequilibrium with the atmosphere. The resulting decrease in atmospheric CO₂ is quite slow; the time constant is probably of the order of a few hundred years³. The Antarctic surface water is flushed every few years by the circulation, but for every turnover, the water takes up only a small part of the atmospheric CO₂ so that the time constant for CO₂ removal is about 100 times the water residence time.

The result found by Peng and Broecker is bound to be somewhat model dependent. Indeed, Joos *et al.*⁴, using a different circulation pattern through the Antarctic surface region and larger surface area, find that the disequilibrium between the

atmospheric and Antarctic surface water p_{CO_2} is maintained at a larger value. The consequent reduction in the rate of increase of atmospheric CO₂ is also somewhat greater.

Surely in looking at how to tackle the threat of greenhouse warming, we should not focus on which model of iron fertilization gives the best result (the possibility of undesirable ecological effects put to one side for the moment), but rather we should worry about the increasing rates of fossil-fuel consumption. The total scale of fossil-fuel reserves is not known with any certainty, but most estimates are between 6 and 12 times the present amount of carbon in the atmosphere as CO₂, mostly as coal⁵. Barring increased uptake by the terrestrial biosphere, which seems unlikely because of deforestation, 70–80 per cent of this carbon will accumulate in the atmosphere if it is burned over a period of a few hundred years, regardless of the actual history of the CO₂ generation over this period⁶. When we have consumed most of the fossil fuel, the atmosphere will have reached a level of at least 1,500 p.p.m. from its present level of around 345 p.p.m.: at this point it may not matter whether a sequestering effort reduced the atmospheric CO₂ by 30 p.p.m. or 300 p.p.m.

However, exactly when the maximum CO₂ level will be reached can vary greatly. For example, a slowly growing production of CO₂ may result in only a doubling of the atmospheric content by the year 2100, but expansion of CO₂ production at the pre-1973 rate of 4.5 per cent a year could lead to levels over 1,200 p.p.m. by then⁶. Because fossil fuel is a finite resource, we will need to find an alternative within the next two or three centuries (sooner if substantial consumption of coal is to be avoided). The slower that fossil fuel is burned in the meantime, the better. Neither iron fertilization nor any other method of CO₂ sequestration can be considered a serious alternative. □

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In two minds

EACH hemisphere of the human brain has its own specialism. The left hemisphere (which dominates the right side of the body) is good at language and logic; the right hemisphere (dominating the left side) has better spatial awareness and emotional intuition. Daedalus points out how our view of a knotty problem tends to shift cyclically back and forth every few hours or so. This, he says, is our two hemispheres looking at the matter in turn.

He relates this alternation of mood to our nostrils, which have to warm and humidify the air before it reaches our lungs. It's a hard job. It can drain 5–15 watts of heat continuously from the warming and wetting surfaces, and the nostrils tend to open alternately, a few hours at a time. (You notice this when you have a cold.) Each hemisphere of the brain puts out only about 10 watts of heat. The active nostril, draining heat copiously from the hemisphere close above it, must cool it strongly. The brain is very sensitive to temperature, growing feverish in the warm and sleepy in the cold. So as each nostril opens up in turn, the hemisphere on that side grows sleepy, and our mood swings accordingly.

In this connection, Daedalus recalls the traditional yoga exercise of breathing in through one nostril and out the other. The nostril of inflow gets no relief from warm exhaled breath, and must cool its hemisphere very strongly — a subtle yogic way of opening the mind to new perceptions. Daedalus's 'psychonasal air-conditioner' provides each nostril with its own air, of defined temperature and humidity. It can fire up or chill out either hemisphere at will. Plumbed into the prototype, DREADCO volunteers are tackling the usual psychiatrists' battery of insulting questions and emotional ordeals. With its aid, the sternest logicians are enjoying flights of fancy, while poets buckle down to mathematical proofs.

The final 'Psyconditioner' will be a special hat with concealed plumbing and heat-pump, and integral nose-piece. By controlling the wearer's nostril-air, it will expand his consciousness under full control. Modern, highly verbal society tends to put the right hemisphere at a disadvantage; but the Psyconditioner will allow anyone to get in touch with his intuitive nature. Its wearer will be able to look at a problem first logically, then intuitively — then (with the aid of a special superheater) with fevered imagination, and even with fevered logic. At a picture-gallery or social function he will engage his right hemisphere, while the small print of an insurance-contract will engross his left one. The most inhibited or undisciplined wearer will be able to experience previously suppressed aspects of his personality — and suppress them again as necessary.

David Jones

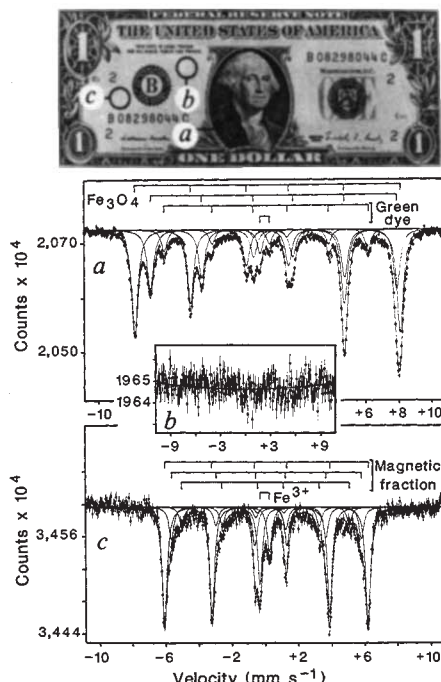
Mössbauer test for forgery

SIR—The relatively high concentration of iron-containing pigments in printers' ink and the specificity of their Mössbauer spectra indicate that Mössbauer spectroscopy could be used successfully for rapid checking of bank-note forgeries. The spectrum from the dollar note in the figure shows that Washington's lapel is printed in an ink whose main pigment is Fe_3O_4 . The black iron oxide Fe_3O_4 is a specific, modern pigment which is not commonly used as a painting material¹. Its strong magnetic properties make dollars 'magnetic'^{2,3}. The ratio I_B/I_A of the two sextet intensities is specific and does not correspond to that of stoichiometric magnetite. X-ray fluorescence shows that parts of the iron atoms in B-sites are replaced by other atoms.

The figure shows a Mössbauer spectrum obtained from a region of green dye only. The Fe^{3+} ions are in several non-equivalent surroundings in the magnetic fraction and also in a paramagnetic compound. The spectrum in *b*, taken from a colourless region, shows that filaments used in the bank-note paper have no iron-containing compounds.

We have not yet studied the iron-containing dollar pigments in detail, but we can say that Mössbauer spectroscopy could become a powerful legal tool in the identification of fakes and forgeries. This conception is based on the pigment's Mössbauer-spectra specificity. Keisch has proposed¹ a similar technique for a similar problem, the investigation of iron-containing pigments used in paintings.

Development of the method as a tool



Mössbauer spectra of a dollar bill. The three spectra *a*, *b* and *c* come from the marked regions of the bill.

for forgery detection requires first, Mössbauer spectroscopy of authentic bank-notes of different values printed in different years; and second, the same investigation on proven forgeries. We would like to appeal for collaborators in our attempts to develop the method. Such sponsors need not to worry about their money: the method is non-destructive.

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Still hope for malaria vaccine?

SIR—Malaria, a life-threatening disease caused by protozoan parasites of the genus *Plasmodium*, affects millions of people in the tropics annually. Resistance of the parasites to a wide range of drugs is now commonplace and spreading. In the search to improve the health of those at risk, vaccines are potentially significant components of an integrated worldwide strategy for malaria control. Gillett's suggestion in Scientific Correspondence (*Nature* **348**, 494; 1990) that this research effort is flawed, and has misunderstood or neglected the problems of genetic diversity generated by sexual reproduction of the parasite, is incorrect. Those involved in the search for effective vaccines have long been aware of the problems posed by polymorphism, not only of the parasite, but also of the mosquito vector and man. The problem of genetic diversity is not exclusive to antimalarial vaccine development: variability in parasite sensitivity to drugs and vector resistance to insecticides have been reported for many years, and significant effort is correctly being addressed to the problem in all three areas.

Certainly, malaria parasites undergo obligatory sexual reproduction to complete the life cycle, but Gillett may have failed to recognize that they are genetically haploid for all stages of the life cycle found in man, and are diploid only in the zygote and ookinete stages in the mosquito midgut. Therefore his implication that the "maintenance and increase in heterozygosity" confers advantage to the parasite is largely irrelevant. Clearly, sexual reproduction allows the generation of new phenotypes by both inter- and intragenic recombination. It must be recognized,

however, that mutation can also readily generate polymorphism in the form of both vaccine-resistant (D. E. Hudson *et al. J. molec. Biol.* **203**, 707; 1988) and drug-resistant parasites.

Solutions to the problems raised by antigenic diversity could come from various well-established and widely published observations. (1) Within any one protein antigen there are both variable and conserved amino-acid sequences. Other constraints permitting, conserved epitopes that are the targets of protective immunity are clearly preferred. (2) For some antigens, functional constraints on the molecule may preclude variation in B- or T-cell epitopes. (3) Antigens in the sexual stages and the zygote not naturally expressed in the vertebrate host show very limited polymorphism. In the light of this discussion it is perhaps ironic to record that these antigens induce a transmission-blocking immunity which attacks, in the mosquito gut, the very stages of sexual reproduction that generate genetic diversity and which are diploid. (4) Attempts are now being made to control by immunization the disease state induced by secreted parasite antigens. The immunity generated need not be anti-parasitic, and the target antigens therefore not subject to the same selection pressures.

Significant progress has been made in our understanding of immunity to malarial parasites. Very successful early laboratory studies produced effective immunity with blood-stage parasites, sexual stages, sporozoites or zygotes, and thus provided an exciting basis for today's research. It has long been appreciated that these early studies used parasites of limited diversity in hosts of restricted genotype. To extend these investigations to the point where immunity can be induced in genetically diverse human populations against polymorphic populations of parasites will require extensive and careful study, but this study must be based upon a secure and informed understanding of the biology of the parasite and its hosts.

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SIR—Gillett¹ expresses an important and valid concern that sexual reproduction in parasites, with its potential for generating

genetic heterogeneity, will preclude the development of effective vaccines. This possibility is not neglected, but rather shared by everyone developing vaccines against any living pathogen. I fear, however, that Gillett's argument at times oversimplifies the problem. Sex is a very good, but not the only, mode of generating diversity. For viruses, prokaryotes and eukaryotes alike, genetic variation as a result of acquiring foreign DNA is relatively common. For example, each year epidemics with antibiotic-resistant bacteria result from transmission of genetic material between different species.

Many parasites have sexual and asexual stages of reproduction, and at times the heterogeneity as well as the selective pressure can be found during the asexual stages. Selection for drug resistance and antigenic variation occurs in the asexual blood stages of *Plasmodium* species. The genome of *Plasmodium* is known to be remarkably flexible in the asexual stages² so that genetic variation need not be the result of sexual recombination. Further, it is not clear that trypanosomes have a

sexual stage, yet a single asexual individual is equipped to present more than 1,000 variants. For most important bacterial and viral pathogens no vaccine exists despite the absence of sexual reproduction in these organisms.

The existence of a sexual stage in itself does not make vaccine development any more difficult. Sexual reproduction in a parasite does not necessarily result in an impossible array of antigenic types. Theoretical considerations aside, no³ or few⁴ antigenic differences are noted between geographically isolated strains of *Schistosoma mansoni*. Because most helminths do not multiply within the definitive host, they present a different kind of problem for host defence mechanisms than either prokaryotes or protozoa. Limited antigenic variation has not been an obstacle to the development of successful vaccines against polio, influenza virus, meningococcus and the pneumococcus.

The absence of sexual reproduction does not guarantee success in vaccine development, similarly, its presence does not guarantee failure. It is clear that

development of vaccines against parasites will not be easy, however: their means of reproduction may not present the main obstacle so much as their complexity. The ability of parasites to present complex surfaces, to repair damage and to otherwise tolerate noxious attacks by their host may be the truly limiting factor. Therefore, the merits and demerits of vaccine strategies compared to other methods of control must be weighed for each pathogen and should not be based on a single aspect of their biology.

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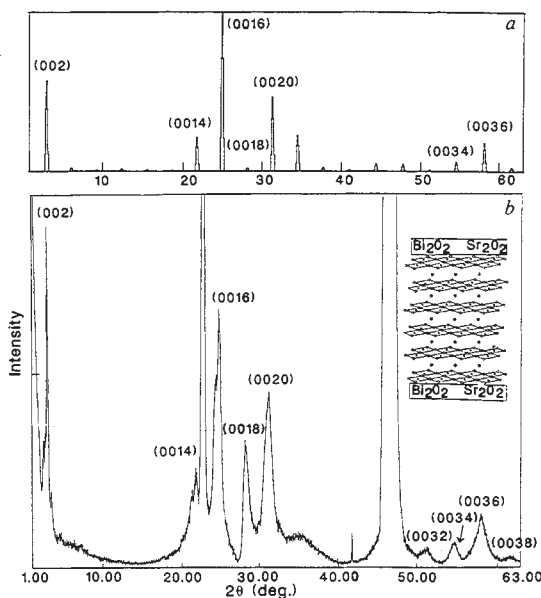
New cuprate superconductors

SIR—In the high-temperature superconductors $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$ and $\text{Tl}_2\text{Ba}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$ (ref. 1), the basic structural units are layers of $\text{Ca}(\text{Sr})\text{CuO}_2$ and the $n=1$ compound. For example, the combination of one CaCuO_2 layer with $\text{Bi}_2\text{Sr}_2\text{CuO}_6$ ($n=1$) makes $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ ($n=2$), two layers of CaCuO_2 make $\text{Bi}_2\text{Sr}_2\text{Ca}_2\text{Cu}_3\text{O}_{10}$ ($n=3$), and ($n-1$) layers gives $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$. For this reason, $\text{Ca}(\text{Sr})\text{CuO}_2$ has been called "the parent structure of high-temperature superconductors"². We have put this concept into action by depositing layers of $\text{Ca}(\text{Sr})\text{CuO}_2$ of desired n on a substrate and periodically inserting a layer of $\text{Bi}_2\text{Sr}_2\text{CuO}_6$. We do this using the technique of laser molecular-beam epitaxy^{3,4}, and we have succeeded in synthesizing thin films not only of $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$ with $n=1-5$, which have already been reported³, but also of the $n=6$, 7 and 8 compounds.

In the experiment we report here, two targets, $\text{Ca}_{0.86}\text{Sr}_{0.14}\text{CuO}_2$ and $\text{Bi}_2\text{Sr}_2\text{CuO}_6$, are alternately ablated by a pulsed ArF excimer laser with appropriate duration, and the layers are deposited on a $\text{SrTiO}_3(100)$ substrate at 500–630 °C in oxygen gas at a pressure of 6×10^{-3} torr.

The first step in the synthesis of any member of the $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$ family is to deposit layers of $\text{Ca}(\text{Sr})\text{CuO}_2$ with the c axis perpendicular to the substrate surface. The partial substitution of cal-

cium by strontium is necessary to obtain smooth, c -axis-oriented layers. (The orientation is confirmed by X-ray diffraction



X-ray diffraction pattern of the $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$ film with $n=6$. The calculated pattern (a) based on the structure in the inset ($\text{Bi}_2\text{Sr}_2\text{Ca}_6\text{Cu}_7\text{O}_{26}$) coincides with the observed X-ray diffraction pattern (b).

peaks at $2\theta = 27.5^\circ$ (001) and 58.4° (002).

The next step is the insertion of $\text{Bi}_2\text{Sr}_2\text{CuO}_6$ layers as required, to produce the different n values of $\text{Bi}_2\text{Sr}_2\text{Ca}_{n-1}\text{Cu}_n\text{O}_{2n+4}$. The number of $\text{Ca}(\text{Sr})\text{CuO}_2$ layers sandwiched by $\text{Bi}_2\text{Sr}_2\text{CuO}_6$ layers can be controlled by a thickness monitor⁵ and confirmed by the oscillation in intensity of reflection high-energy electron diffraction peaks. Large- n compounds are formed

simply by increasing the number of $\text{Ca}(\text{Sr})\text{CuO}_2$ layers sandwiched by the $\text{Bi}_2\text{Sr}_2\text{CuO}_6$ layer. The X-ray diffraction pattern of the newly formed $\text{Bi}_2\text{Sr}_2\text{Ca}_6\text{Cu}_7\text{O}_{26}$ ($n=6$) compound is shown in the figure. Not only the position of the (002) peak at $2\theta=3.0^\circ$ but also the total X-ray pattern coincides with that calculated for the $n=6$ compound ($c=58.8 \text{ \AA}$). In a similar manner, the $n=7$ and 8 compounds can be formed by this technique.

We believe that the inability, until now, to synthesize the $n=6-8$ compounds has been due to insufficient care in growing the $\text{Ca}(\text{Sr})\text{CuO}_2$ parent material. This basic concept and the synthesis technique described here should be useful in the design and creation of other new layered compounds.

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Correction

In the Scientific Correspondence from Kennedy *et al.* 'Striate cortex periodicity' (*Nature* **348**, 494; 1990) reference 5 should have read: (C. Dehay, G. Horsburgh, M. Berland, H. Killackey and H. Kennedy, manuscript in preparation). □

A hard day's night

A. Pais

The Scientific Letters and Papers of James Clerk Maxwell. Volume 1, 1846–1862. Edited by P. M. Harman. Cambridge University Press: 1990. Pp.748. £125, \$195.

JAMES Clerk Maxwell's first biography appeared in 1882, three years after his death at the age of 48. (In 1969 it was reissued by the Johnson Reprint Corporation.) It was written by the Reverend Lewis Campbell, Maxwell's life-long friend ever since their school days (1841–47) at the Edinburgh Academy, together with William Garnett, Maxwell's Demonstrator at the Cavendish Laboratory in Cambridge. This charming and instructive book is still the best source for Maxwell's personal life, but leaves much to be desired with regard to a sketch of his scientific contributions. The book also contains some, but not all, of Maxwell's correspondence.

In 1890 the Cambridge University Press published *The Scientific Papers of Maxwell* (reissued by Dover in 1952). It contains most but not all of his technical papers: shorter publications were omitted.

Now we have before us the first volume of Maxwell's scientific letters and papers, currently known to exist, edited by Peter Harman. Taken together with the two books mentioned before, this excellent, thoroughly documented and handsomely produced work, and its promised sequels, will fill the basic needs of present and future Maxwell students and will introduce him to a broad audience as "a man of wide reading and deep learning, a scholar as well as a scientist."

The regrettable repetition of the term 'scientific papers' in the title of Harman's book, also used in the 1890 book mentioned above, fails to emphasize an essential difference between these two works. In the earlier work, the main published papers are reproduced, none of which reappears in the new volume. One novel feature of Harman's book is that there one finds unpublished drafts of many of these same papers, a great help in obtaining new insights into the evolution of Maxwell's thinking. Not all drafts led to corresponding publications, but where that is the case one always finds a helpful note referring back to the earlier book.

The present volume covers the years 1846–62, thus spanning Maxwell's last schoolyear, his university studies in Edinburgh (1847–50) and Cambridge (1850–54), and his professorships at

Marischal College, Aberdeen (1856–59) and, in part, King's College, London (1860–65). It opens with a draft of Maxwell's paper (1846) on oval curves, written while still at school. As it ends, in 1862, we are still two years away from the publication of his fundamental memoir on electromagnetic field theory. Yet we learn

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James Clerk Maxwell — a scholar and a scientist.

that he already had many of its main ideas clear in his mind. Thus Maxwell to Faraday, 19 October 1861: "We have now strong reason to believe, whether my theory is a fact or not, that the luminiferous and the electromagnetic medium are one . . . I avoided all the old traditions about forces acting at a distance" (pages 686, 688). To William Thomson (Kelvin), 10 December 1861, he writes of "the nearness between the two values of the velocity of propagation of magnetic effects and that of light" (pages 695, 690). Maxwell discussed this last point quantitatively in part III of his article On physical lines of force published in early 1862 (see *The Scientific Papers of Maxwell*, page 489). This major paper is not found in Harman's book, because apparently no drafts are extant. The moral of this is that Harman's book will be appreciated better with a

copy of the earlier work at hand.

In the nine years following 1846, Maxwell published only four papers, including his first on physics (1849), on equilibrium of elastic solids (Harman, pages 120–185). He had, however, already written a manuscript on the effects produced by polarized light (Harman, pages 99, 101), work that was influenced by his acquaintance with old William Nicol of Edinburgh (he of the prisms).

In 1855 another topic that had long interested him came to fruition when he founded the science of quantitative colorimetry. "My theory only professes to prove that there are three and only three [primary colours] and does not define them" (Harman, page 568). He pursued this subject for several years (Harman, pages 600–680), not only as theoretician but also as consummate experimentalist (a combination not that uncommon in those days). His letters to colleagues show him having a great time with rotating disks and tops, and reporting important contributions to the origins of colour blindness, repeatedly requesting them to send him colour-blind students for testing.

Also beginning in 1855 Maxwell turned his attention to "The stability of the motion of Saturn's rings" (Harman, page 438), the subject for the Adam's Prize of 1856, awarded to him in 1857, and keeping him busy for several years thereafter. In this work we encounter Maxwell for the first time as the mature, powerful theoretician.

The main other drafts found in Harman's book deal with Faraday's lines of Force (pages 337–371). The book concludes (page 713) with a draft on motions and collisions of particles. The corresponding paper (see *The Scientific Papers of Maxwell* Volume 1, page 377) on the Maxwell distribution of velocities, ingenious in concept and results, flawed in execution, marks the beginnings of Maxwell's role in statistical physics, along with colour vision and optics and with electromagnetism the third main theme in his *oeuvre*.

Of particular interest among hitherto unpublished material is Maxwell's inaugural lecture at Aberdeen (page 419) in which he urges study of the world so as "to subdue the earth and have dominion over the creatures." In another lecture (page 542) he tells students "to know, to submit to, and to fulfill the laws which the Author of the Universe has appointed." At Aberdeen he taught 15 hours a week (page 557). To Campbell: "The total oblivion of [pupils] for definite intervals is a necessary condition for doing them justice at the

proper time" (page 712). I may interject here a story about Maxwell's teaching told to me by Kapitza, who had it from Horace Lamb, who had followed Maxwell's courses in Cambridge. According to Lamb, Maxwell would often make mistakes in his lectures. Then he would calmly say: something is wrong, then search the blackboard, then calmly say: here is my mistake. That behaviour by a great man had been truly inspiring, Lamb had said.

My greatest pleasure came in reading Maxwell's letters (those to him are merely listed in an appendix). Scientifically, the most important are those to Stokes and Thomson. Letters to friends reveal more about Maxwell the man. Just a few samples: "After breakfast is the best time for reading Greek and Latin, because if I read newspapers or any of those things then it is dissipation and ruin" (page 193). On studying in Cambridge: "He starves while being crammed. He wants man's meat, not college pudding" (page 208). On his impending marriage: "Working together and thinking together we shall both be free" (page 586). On Faraday: "What a painful amount of modesty he has" (page 556).

It is fitting to conclude this review of a fine book with these words from a great man (page 583): "Let us work while it is day, for the night is coming and work by day leads to rest by night." □

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Umbugology and ditchwateristics

D. V. Lindley

The Taming of Chance. By Ian Hacking. Cambridge University Press: 1990. Pp.264. Hbk £27.50, \$44.50; pbk £9.95, \$14.95.

THERE are two distinct features of our appreciation of the world that obey the same mathematical rules. The first is chance, a phenomenon associated with repetitions in which a stable frequency is apparent. Tosses of a coin provide the simplest example; the normal distribution of people's heights is a little deeper. The second is belief, describing one's understanding of an uncertain world. You have a belief that it will rain tomorrow, or that the seas will rise as a result of the greenhouse effect. The mathematics common to chance and beliefs is the calculus of probability. That this is true for chance is admitted by all. There is some dispute over whether beliefs combine probabilistically. There are adherents of

fuzzy logic, of chaos theory, of belief functions, and even statisticians have their doubts.

Ian Hacking's concern in *The Taming of Chance*, except briefly in a final chapter dealing with the work of C. S. Peirce, is with chance. He carefully and entertainingly shows how the idea developed as a result of the masses of statistics produced, more especially in Prussia and France, leading to the discovery of statistical laws and their incorporation into the mainstreams of knowledge. Related to this is the realization that the physical world is not purely deterministic, but also has chance features so that probability pervades both science and society. "Probability is, then, the philosophical success story of the first half of the twentieth century". This is an exaggeration whilst statisticians persist in their refusal to speak of the probability of a hypothesis being true, but is correct when applied to chance, as Hacking admirably demonstrates.

Hacking's argument is supported by a vast number of references to statistical work and the interpretations put upon it. The notes run to 40 pages. In addition, there are often amusing footnotes, from one of which the title of this review is taken (from Dickens) — he seems to have read everything. At times I found his style overwhelming in its complexity, as in "probability is a Janus-faced mid-seventeenth-century mutation in the Renaissance idea of signs." Fortunately, the list of contents, running to five pages, presents the main thesis with real clarity and provides the reader with an excellent summary.

The central thesis is surely correct. Chance and its laws did develop from collections of data. They have affected our understanding of the world even in situations like quantum physics, far removed from the original observations of suicide rates. But is this thesis original? Have we not known this for a long time and more recently cursed it for making people think that probability is essentially based on frequency? Certainly the documentation provided here is extensive, but the key issue today is whether the laws of chance are those of beliefs. Beliefs are often formed by frequency data, as when you buy an annuity whose calculation is based on statistics. But they need not be, as when a man believes he will live longer than the insurance company thinks because he comes from a long-lived family and is happily married in social class 1. And what use is the thesis? Certainly the presentation here is interesting and lively and provides excellent entertainment for a scientist who takes his relaxation seriously. But will it help him to be a better scientist in the laboratory or the field next morning? I think not. The history of any branch of science suffers from this inadequacy that it rarely helps us to be better

scientists. We are not alone in this; a study of Plato is held to be useful to a politician. If so, it is a sad reflection on the progress of political science over 2,000 years. Instead of wondering whence probability came, it would be better to understand its position today as Hacking did in his earlier *Logic of Statistical Inference* (Cambridge University Press, 1965). □

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■ See also *The Empire of Chance: How Probability Changed Science and Everyday Life* by Gerd Gigerenzer, Zeno Swijtink, Theodore Porter, Lorraine Daston, John Beatty and Lorenz Krüger. Published by Cambridge University Press. Price is £32.50, \$44.50 Hbk; £12.95, \$16.95 pbk.

Culture shock?

Andrew C. Street

Gaijin Scientist, Science and Technology Group. British Chamber of Commerce: 1990. Pp. 129. £10.

FOR scientists contemplating working in Japan *Gaijin Scientist* is an essential read; *gaijin* being most conveniently translated as foreign. The authors provide a substantial introduction on how to find a research post in either the private or public sectors and on what to expect when one arrives. One strength of the book is the numerous, informative quotes throughout the text, by more than 20 British scientists and engineers recalling their experiences in Japan.

The principal message is that opportunities for carrying out top-class work do exist in Japan, but great care should be taken in choosing where and with whom to work. The authors compare and contrast the standard of facilities found in the universities and national laboratories, and vividly highlight the differences in work ethic that can exist between a Japanese academic group and its British counterpart. The book also contains a comprehensive list of organizations which offer or coordinate schemes for research posts within the public sector.

The authors provide the reader with an understanding of the fundamental differences in attitudes towards work and career which exist between Japanese and British companies. Extracts from the case studies of scientists and engineers working in Japanese companies provide an excellent insight into 'company life'. In addition to reviewing the typical terms and conditions of employment within a company the book also provides a non-exhaustive list of companies known to be employing *gaijin* scientists.

The text serves as a mine of information on things which should be done to ensure

a smooth transition into the Japanese working and living environments. Allowing time for obtaining the necessary visa, and making ample preparation for speaking some Japanese are probably two of the more obvious recommendations. But a prospective *gaijin* scientist may well be surprised to read that one option of accommodation for a single company employee is the confined and closely supervised company dormitory (a high-voltage culture shock!), and that Japanese pillows may be stuffed with rice.

Valuable advice is also given for arrival in Japan, including, for example, alien registration, and on the use of personal seals (*hanko*) rather than signatures. Differences between the social life in Japan and the West, and advice on how to overcome them, is pertinently described with the aid of the scientists' quotations, as well as what to do when one's research contract is over — to remain in Japan, or to return to find work in Europe. □

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Matching bones to niches

R. D. Martin

Animal Lifestyles and Anatomies: The Case of the Prosimian Primates. By Charles Oxnard, Robin Crompton and Susan Lieberman. University of Washington Press: 1990. Pp. 174. \$35.

THE extensive multivariate studies conducted by Charles Oxnard on dimensions of the locomotor skeleton of primates are well known, having been covered in his previous books: *Form and Pattern in Human Evolution* (University of Chicago Press, 1973), *Uniqueness and Diversity in Human Evolution* (University of Chicago Press, 1975) and *The Order of Man* (Yale University Press, 1983). The laudable basic tenet of these published studies, reflecting the early influence of Solly Zuckerman (now Lord Zuckerman), is that quantification is essential for effective comparison. Until recently, however, such broad multivariate studies of the skeleton have not been matched by an equivalent analysis of quantitative data on the natural ecology and locomotor behaviour of primates. It has therefore been difficult to conduct a reliable assessment of quantitative variation in skeletal features in relation to natural patterns of locomotion. In *Animal Lifestyles and Anatomies: The Case of the Prosimian Primates*, Oxnard has been joined by Robin Crompton and Susan Lieberman, who have together provided a comple-

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Terrestrial macaques — does body size influence mode of locomotion?

mentary quantitative investigation of primate lifestyles. In the terminology of the book, the field of 'morphometrics' has been married with that of 'niche metrics'. The result is an impressive survey that examines the degree of correspondence between assessments of natural ecological patterns and multivariate abstractions of morphological variation in the skeleton.

Although the book is confined to the prosimian primates, the rich array of species considered provides an ample basis for comparative purposes and (as the authors note) the approach may be extended to simian primates as a second step. Following a brief presentation of results from Oxnard's previous multivariate studies, the core of *Animal Lifestyles and Anatomies* consists of a valuable, well-written review of the locomotor behaviour and general ecology of individual prosimian species. Although much of this necessarily draws on information available in the literature, it has obviously benefited greatly from the practical experience afforded by Crompton's pioneering field studies of bushbabies and tarsiers. In these studies, Crompton neatly followed up suggestions originally made by Alan Walker regarding the need for detailed quantification of primate locomotion under natural conditions. In fact, given that the tack taken by Oxnard has hitherto had little direct interaction with the Napier/Walker approach to the investigation of primate locomotor adaptation, the meeting of these two traditions can be seen as a very positive development in *Animal Lifestyles and Anatomies*.

Another important element has been contributed by Susan Lieberman, who — as a tropical ecologist — injected expertise in terms of examining overall ecological

patterns. She was presumably responsible for the decision to use polar coordinate plots for the presentation of data on habitat, locomotion and diet for each species. Given a little practice, these striking diagrams can serve a useful role as succinct visual summaries of overall patterns, though I must admit that I would have felt more comfortable with histograms. Use of a circular plot tends to imply continuity between adjacent categories and it is hence somewhat unsettling to find the four dietary categories wedged between 'scurrying' and 'running leap' in the bottom right-hand sector. The text is also enhanced by numerous well-chosen line-drawings of prosimians, most notably by Erika Oller. For many primatologists, the combination of plenty of attractive and informative illustrations with concise, accurate surveys of the lifestyles of individual prosimian species will provide a compelling reason to acquire this book.

After presenting patterns for individual prosimian species, the authors cluster them and then discuss the groups that emerge. Having recognized clusters on the basis of patterns identified in habitat, locomotion and diet, it is possible to apply canonical analysis to examine factors that influence clustering. One such analysis shows that body weight exerts a distinct, though rather complex, influence. Strong effects are also exerted by taxonomic grouping and by ecological categories (habitat, locomotion and diet). There is almost complete separation between the effects of habitat and locomotor variables, whereas the four dietary categories all exert markedly divergent effects. In the final chapter, it is demonstrated that the overall clustering of prosimian species on the basis of lifestyles quite closely matches that obtained from Oxnard's multivariate

analysis of morphometric data. But this finding is tempered by the fact that Oxnard's analysis was conducted only at the generic level and therefore missed the major differences that exist between individual species within genera. Field data have revealed, for example, that there are important locomotor differences between species of the genus *Galago*.

There is no doubt that this book represents a significant new contribution to the literature on primate locomotion. At the same time, it must be emphasized that even this combined approach has its limitations. The various clustering procedures presented for both morphometrics and niche metrics are simply methods of data presentation. In themselves, they do not lead us any further along the road to analysis and interpretation. It is difficult to see, for instance, how the approach presented can be applied to the interpretation of fossil primates; the morphometric analyses are too abstract and are confined to the generic level, while niche data are simply not available at the requisite level of refinement for fossil species. It is, indeed, my personal conviction that multivariate studies of prosimian locomotion will never lead to real understanding until the influence of body size has been explicitly taken into account. In this book, the influence of body size is not even mentioned until page 124, where it is then stated that this factor "cries out" for analysis. It is therefore all the more surprising that the authors have virtually omitted any mention of the abundant recent literature on allometric scaling of locomotor and dietary variables in primates.

The basic point is this: if we compare (say) locomotor patterns of a small-bodied arboreal macaque with those of a larger-bodied terrestrial macaque and find differences, to what extent do these differences reflect a real divergence between arboreal and terrestrial locomotor adaptation and to what extent do they merely reflect the effect of body size? The problem can only be tackled by restricting comparisons to species of the same body size or by applying an appropriate procedure to 'remove' the size effect. My own interest in this question was in its infancy when I took part in a conference at the

Zoological Society of London in 1972. After Oxnard had presented his paper on morphometrics, the late Nigel Barnicot asked him to explain how he dealt with the confounding influence of body size. Oxnard's answer was that he believed that his results had not been heavily biased by size effects. One of the major conclusions to emerge from *Animal Lifestyles and Anatomies*, unheralded by the authors, is that this reply was probably in error. The multivariate pattern that emerges from niche metrics is convincingly shown to be influenced by body size and that pattern is then shown to be closely similar to that obtained from Oxnard's multivariate analysis of morphometric data. It therefore presumably follows that the latter is also influenced by body size (QED). Perhaps when morphometrics and niche metrics have been joined by allometrics we will finally achieve the understanding of primate locomotor adaptation that we are all seeking. □

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To boldly go . . .

Nicole Borderies

Uranus the Planet, Rings and Satellites. By Ellis D. Miner. Horwood: 1990. Pp. 334. £35.50.

THE Voyager missions to the outer planets of the Solar System were characterized by the ability of the Jet Propulsion Laboratory (JPL) engineers to respond successfully to all the challenges that they faced, and by the wealth of scientific data obtained during each encounter. The Voyager spacecraft had been designed to fly by the systems of Jupiter and Saturn. Only after the successful encounter of Voyager 1 with Saturn and Titan, were Voyager project personnel given permission by NASA Headquarters to target Voyager 2 for a Uranus trajectory. Voyager 2 flew by Uranus on 24 January 1986 and continued on to an encounter with Neptune on 25 August 1989, 12 years after its launch.

Ellis Miner, who has served as Assistant Project Scientist since shortly after the launches of Voyagers 1 and 2 in the late summer of 1977, presents an insider's point of view of these missions. This, together with his goal of comprehensiveness and the historical perspective he adopts, are the strongest characteristics of this book. Remarking that the extraordinary successes of the Voyager missions cannot be attributed to good luck, Miner recalls how the Voyager missions came to life. In 1966, Gary Flandro, a JPL and Caltech scientist, showed that gravity

assist (the phenomenon by which a spacecraft passing a planet in certain conditions increases its velocity) could be used to send a single spacecraft to several planets of the outer Solar System. In 1970, JPL proposed a 'Grand Tour' mission to NASA, which involved sending two spacecraft to Jupiter, Saturn and Pluto, followed by two more spacecraft to Jupiter, Saturn and Uranus. In response to a request from NASA headquarters for a less expensive version of the 'Grand Tour', JPL proposed a two-spacecraft Mariner Jupiter-Saturn (MJS) mission. Because the MJS spacecraft was developing into something quite different from the previous Mariner spacecraft, it was called Voyager.

Miner's insider view also puts him in a good position to describe the technical aspects of the missions. These include for instance: the instrumentation for the 11 scientific investigations; the launches of the two spacecraft; the problems along the way; the enhancement of the capability of the Deep Space Network to detect Voyager's signal from Uranus; the reprogramming of the computers on board Voyager 2. Another advantage of Miner's unique involvement in the Voyager project is that it gives him a deep understanding of how the project was managed. He explains how the major scientific goals of the Uranus encounter were selected and how the planning was done. He even shows organization charts with the names of the people involved in the project at the time of the encounter with Uranus.

Miner's book is also about science. Here again, his goal is to be comprehensive. The discovery of Uranus by Sir William Herschel is recounted, as well as the discovery of the satellites, and more recently that of the rings by J. L. Elliot and his colleagues. Our pre-Voyager knowledge of the system of Uranus, its rings and its satellites, is described towards the beginning of the book, whereas the state of knowledge after Voyager is given in the last chapters. This information is classically divided into 'Interior', 'Atmosphere', 'Magnetosphere', 'Rings' and 'Satellites'. The description of the new data obtained by Voyager is fairly detailed. Due to the intent of the author to write a book understandable by non-scientists and to the fact that the detailed study of the Voyager data has not been completed, the parts on the interpretation of the data have a more sketchy and preliminary character.

I enjoyed reading the book and I think it would appeal to a wide range of readers who would like to have a feel of what it is to be a part of space exploration. □

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The Evolution of *Homo erectus* (Correction)

In Michael Day's review of *The Evolution of Homo erectus* by G. Philip Rightmire (*Nature* 348, 688; 1990) the sentence 'This group of material formed the basis for Louis Leakey's theory that man arose as an Asian species rather than one from Africa', should have read 'It was upon this group of material that was based the theory that man arose as an Asian species rather than one from Africa as believed by Louis Leakey'. This editorial error, for which we apologize, reversed the author's intended meaning; Louis Leakey never believed man to be an Asian species and always favoured an African origin. □

Mitochondrial proteins essential for viability mediate protein import into yeast mitochondria

Kevin P. Baker & Gottfried Schatz

Only five mitochondrial proteins are known to be essential for viability of the yeast *Saccharomyces cerevisiae*; all of them are key components of the mitochondrial protein import system. Other components of this system are not essential for life; they include functionally redundant import receptors on the mitochondrial surface and enzymes acting upon only a few precursor proteins.

MITOCHONDRIA, the organelles of oxidative phosphorylation of eukaryotic cells, also participate in the synthesis of haem, pyrimidines, amino acids and many other key metabolites. Thus, mitochondria are essential to the eukaryotic cell even during anaerobic metabolism¹. Indeed, there is no evidence that eukaryotic cells can reversibly lose them^{2,3}. Mitochondria contain their own genetic system and synthesize some polypeptides (13 in humans), but most of the hundreds of different mitochondrial polypeptides are encoded by nuclear DNA, synthesized in the cytoplasm, and imported into the mitochondria⁴. This protein import is thus indispensable for mitochondrial biogenesis and life.

Protein import machinery

Mitochondria contain an outer and an inner membrane which enclose two separate compartments, the matrix and the inter-membrane space. Most proteins destined to be transported from the cytoplasm into the matrix are synthesized with N-terminal amphiphilic targeting sequences that direct the protein into mitochondria. The precursor protein then binds to the mitochondrial surface and is transported across the outer and the inner membrane at sites where the two membranes are closely apposed. Passage across the two membranes requires that the protein be loosely folded. Once the protein has reached the matrix, its targeting sequence is removed and the mature polypeptide folds into its native conformation^{4,5}. Each of these steps is catalysed by specific proteins. Premature folding in the cytoplasm is prevented by cytosolic 'chaperones'⁶ which selectively bind and stabilize partly folded proteins; specific binding to the mitochondrial surface is mediated by mitochondrial surface receptors; transport across the mitochondrial membranes is catalysed by proteins forming 'protein-transport pores' across these membranes; removal of the targeting sequence is effected by a specific protease; and folding in the matrix is controlled by matrix-located chaperones. The mitochondrial members of this protein family collectively represent the mitochondrial protein import machinery.

The yeast mitochondrial protein import system contains two hierarchies of components: those essential for protein import and life; and functionally redundant, nonessential components. A 'functionally redundant' component can be bypassed because the precursor protein can either interact with components which would normally act later in the pathway, or which operate in parallel with a functionally similar component of overlapping precursor specificity. The existence of functionally redundant import catalysts has hampered identification of these catalysts, because their removal or inactivation may inhibit protein import into mitochondria only partly, or not at all. This problem is particularly acute when studying protein import into isolated mitochondria as the rate-limiting steps with isolated organelles are often different from those in living cells. The yeast *Saccharomyces cerevisiae* is important in assessing the biological relevance of *in vitro* import experiments because it is currently one of the few eukaryotic organisms in which the *in vivo* function

of a protein can be explored by deleting its gene⁷. Our discussion will thus focus on results obtained with yeast, although we will attempt to relate these results to those obtained in other organisms.

Five essential import proteins

The first two mitochondrial import catalysts essential for life were detected by screening a collection of approximately 2,000 temperature-sensitive lethal yeast mutants for accumulation of uncleaved mitochondrial precursor proteins at the restrictive temperature^{8,9}. The two mutants obtained by this 'brute force' screen accumulated the uncleaved precursors outside the inner membrane. The mutations turned out to be in two unlinked nuclear genes which were termed *MAS1* and *MAS2* (for mitochondrial assembly). Different mutant alleles of these two genes were later isolated by another screen¹⁰. *MAS1* and *MAS2* encode the two non-identical subunits of the matrix-located processing protease¹⁰⁻¹³, a water-soluble, metal-requiring heterodimer that removes matrix-targeting sequences from precursors imported into the matrix space¹³⁻¹⁹. The two subunits have similar amino-acid sequences and are synthesized with typical matrix-targeting signals of 19 and 13 residues, respectively, which are cleaved off by the pre-existing, already assembled *MAS*-protease^{11,19}. Disruption of either of the two *MAS* genes is lethal¹⁰⁻¹², but it is not clear why the *MAS*-protease is essential for viability. Removal of the amphiphilic matrix-targeting sequences from precursor proteins could be required to allow either proper assembly of imported proteins or their dissociation from other components of the import machinery. Alternatively, the intracellular accumulation of proteins with amphiphilic targeting sequences²⁰ might poison membrane functions.

The third essential protein of the mitochondrial protein import machinery was identified by screening a collection of temperature-sensitive lethal yeast mutants for assembly of active ornithine transcarbamylase trimers in the mitochondrial matrix²¹. One of these mutants (termed *mif4*, for mitochondrial import function) contained a temperature-sensitive mitochondrial heat-shock protein of relative molecular weight 60,000 (60K). This protein (now generally termed mitochondrial hsp60) had earlier been detected in the mitochondrial matrix of many different organisms²²; its deduced amino-acid sequence is similar to that of the *groEL* gene product, one of the major heat-shock proteins of *Escherichia coli*. Mitochondrial hsp60, like the *groEL* gene product, is a homoligomer composed of 14 subunits which mediates refolding of newly imported mitochondrial proteins. It binds these proteins in the matrix, keeps them in a loosely folded conformation and releases them again in a process requiring cleavage of ATP and at least one other matrix protein²³. Mitochondria of the *mif4* mutant still import proteins at the non-permissive temperature, but cannot assemble them properly. Mitochondrial hsp60 is thus a typical 'chaperone' whose function resembles closely analogous proteins in bacteria and chloroplasts⁶. As proteins imported into the matrix space

in the absence of functional hsp60 cannot generate their native conformation, inactivation of mitochondrial hsp60 is lethal.

A 70K stress protein of the matrix is the fourth essential mitochondrial protein. Its function in mitochondrial protein import was recently determined by crosslinking it to a chimaeric mitochondrial precursor stuck in the import site²⁴, by observing transient association with precursors entering the matrix^{24,25}, and by showing that its heat-inactivation in a temperature-sensitive mutant blocks protein import into mitochondria²⁵. The protein is encoded by the *SSC1* gene and is similar to the *E. coli* *dnaK* gene product except that it is made with a cleaved matrix-targeting signal of 23 residues^{24,26,27}. This 'mitochondrial hsp70' (mhsp70) is found in a complex which contains a stuck precursor as well as the outer membrane protein ISP42 (see below). Pulse-chase studies (U. C. Krieg and P. Scherer, unpublished) indicate that mhsp70 binds imported precursors before hsp60, that the association is transient and that it is disrupted by ATP. Mitochondrial hsp70 thus mediates one of the earliest essential interactions of a precursor in the matrix. This interaction, coupled with ATP-mediated release, could provide the driving force for the transmembrane movement of the polypeptide chain, and might explain why protein import requires nucleoside triphosphate in the matrix^{4,5}. It is not known whether mhsp70 transfers bound precursors to mitochondrial hsp60. Protein import into yeast mitochondria is also accelerated by functionally redundant 70K heat-shock proteins in the cytosol^{28,29}; these are not considered here because they are not mitochondrial proteins (see Fig. 1).

The fifth essential component of the protein import system is a 42K protein³⁰. As it can be efficiently crosslinked to a partly translocated chimaeric precursor carrying a photo-activatable diazirin group, it was termed import site protein 42, or ISP42. ISP42 is an integral outer membrane protein even though the nucleotide sequence of its gene predicts a protein without an obvious membrane-spanning segment or N-terminal mitochondrial targeting signal³¹. It seems to be a key component of the mitochondrial protein import machinery: when expression of the *ISP42* gene in growing yeast cells is repressed, the cells accumulate mitochondrial precursor proteins and cease growth. In agreement with this observation, disruption of the *ISP42* gene is lethal. ISP42 seems to be a subunit of a hetero-oligomeric transport channel for precursors across the outer membrane. This would fit with the transmembrane location of the partly translocated precursor that can be crosslinked to ISP42 and with the observation that overproduction of ISP42 causes it to mislocalize and denature within mitochondria. Indeed, the *Neurospora* homologue of ISP42 (termed MOM38) can be isolated as a complex with several other outer membrane proteins, including two import receptors³². ISP42 is the only mitochondrial membrane protein so far known to be essential for viability.

The five mitochondrial proteins discussed above appear to be part of the indispensable 'core' of the mitochondrial protein import machinery (Table 1). They probably represent com-

ponents of the 'central import pathway' that is followed by all mitochondrial components destined for the matrix. A central import pathway across the mitochondrial outer membrane had been proposed on the basis of inhibition experiments with isolated *Neurospora* mitochondria³³. The genetic studies with yeast support the idea that such a pathway exists and show that it includes soluble matrix proteins.

Functionally redundant receptors

In vitro import of precursors into mitochondria is aided by mitochondrial surface proteins because treatment of intact mitochondria with proteases^{34,35} or antibodies against outer membrane proteins³⁶ inhibits import. However, identification of these putative 'import receptors' has been difficult: genetic screens have failed to uncover yeast mutants with defective import receptors and biochemical characterization has been hampered by the lack of a reliable *in vitro* assay.

Nevertheless, two receptor-like proteins of the yeast mitochondrial outer membrane have now been identified. One of them (MAS70) is a major 70K protein anchored to the outer membrane by a hydrophobic sequence near its N-terminus, exposing a 60K domain to the cytosol³⁷⁻³⁹. This protein belongs to a family whose members contain several 34-residue repeats; the other members function in the control of the fungal cell cycle and in *Drosophila* neurogenesis^{40,41}. If the MAS70 protein of intact yeast mitochondria is inactivated by monospecific IgGs, cleaved by mild trypsin treatment or eliminated by disrupting the nuclear *MAS70* gene, import of several precursors into mitochondria is inhibited by 50–80%, whereas that of others is inhibited only slightly or not at all. A similar protein (MOM72) has been identified as an import receptor in *Neurospora* mitochondria, but its receptor function seems to be limited to a single precursor, that of the ADP/ATP translocator⁴². Disruption of the *MAS70* gene does not kill yeast, but merely slows both mitochondrial protein import *in vivo* and growth on nonfermentable carbon sources at high temperature³⁴. Thus MAS70 accelerates the import of a subgroup of precursors without being essential even for this subgroup. Inhibition experiments with different antisera indicate that precursors whose import is accelerated by MAS70 can also be imported through other, as yet unidentified receptor proteins. MAS70, like MOM72 in *Neurospora*, is enriched in sites of close contact between the two mitochondrial membranes. It may accelerate import by binding precursors and delivering them to the import channel in the outer membrane.

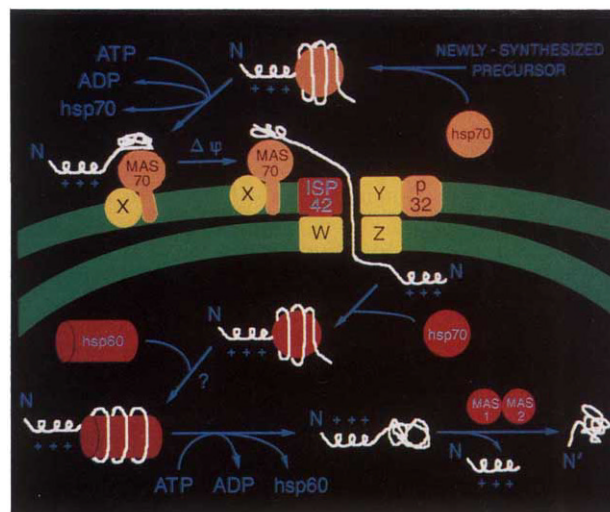
The second import receptor of yeast is a 32K protein that was found with the aid of anti-idiotypic antibodies against a matrix-targeting peptide^{43,44}. The deduced sequence of this protein resembles that of the phosphate transporter of mammalian mitochondria. As the protein forms complexes with mitochondrial precursors, and as antibodies against it partially inhibit import of several precursors into isolated mitochondria, it, too, is probably functionally redundant. Indeed, disruption of its

TABLE 1 Essential and non-essential components of yeast mitochondrial protein import system

Component	Gene	Location	Essential?	Function	Refs
MAS1	<i>MAS1</i>	Matrix	Yes	Removal of matrix-targeting signal	10–18
MAS2	<i>MAS2</i>	Matrix	Yes	Removal of matrix-targeting signal	10–18
hsp60	<i>MIF4</i>	Matrix	Yes	Refolding of imported proteins	21–23
mhsp70	<i>SSC1</i>	Matrix	Yes	Refolding and transmembrane movement of precursors?	24–26
ISP42	<i>ISP42</i>	Outer membrane	Yes	Translocation across outer membrane	30, 31
MAS70	<i>MAS70</i>	Outer membrane	No	Import receptor	37, 38
p32	<i>MIR1</i>	Outer membrane	No	Import receptor	40, 44
Haem lyase	<i>CYC3</i>	Intermembrane space	No	Import of apocytochrome <i>c</i>	47–50
Inner membrane protease I	<i>IMP1</i>	Inner membrane	No	Processing of cytochrome <i>b₂</i> and cytochrome oxidase subunit II	51, 52

Extramitochondrial proteins, such as cytosolic 70K heat-shock proteins, are not included.

FIG. 1 Import of a precursor (white) with a cleavable matrix-targeting signal across the two mitochondrial membranes (green) of yeast. The five essential import components are red, functionally redundant components orange, and hypothetical components yellow. + signs, matrix-targeting signal; X, unidentified redundant import receptors (perhaps the MOM19 homologue of yeast); Y, W and Z, hypothetical additional proteins of the import channels across the outer and inner membrane. See text for further details. The order of events in the mitochondrial matrix is not known, but hsp70 seems to act before hsp60 or the MAS-protease. Import into mitochondria from *Neurospora* or rat liver seems to involve closely similar components; these are listed in Table 2 together with their yeast homologues.



single nuclear gene *MIR1* is not lethal, but renders yeast respiration-deficient. The size and the proposed function of the *MIR1* gene product resemble those of a 29K rat-liver protein which, after reconstitution into liposomes, mediates binding of a precursor protein to liposomes⁴⁵. It is not known whether this protein is the mammalian homologue of the 32K yeast protein. Another putative receptor protein has been identified by biochemical means in isolated *Neurospora crassa* mitochondria⁴⁶. The protein has a molecular weight of 19K and promotes mitochondrial import of many, but not all, precursors. Its yeast homologue has an apparent molecular weight of 17K (ref. 32). Its function *in vivo* and its requirement for viability have yet to be established.

Other components

Some mitochondrial proteins mediate the import of only a few precursors or of a single precursor. Inactivation or loss of these proteins prevents import or maturation of the corresponding precursors, but does not effect global protein import or viability. The best-studied example of this group is cytochrome *c* haem lyase which catalyses the covalent attachment of haem to apocytochrome *c*. Haem lyase is located in the space between the two mitochondrial membranes⁴³. Its inactivation by haem analogues (in *Neurospora crassa*) or by mutation of its structural gene (in yeast) prevents import of apocytochrome *c* into mitochondria, presumably because the covalent attachment of haem traps the protein in the intermembrane space^{48,49}. Cytochrome *c* haem lyase is not required for import of other mitochondrial precursors, including the precursor of apocyto-

chrome *c*₁ (ref. 50). Yeast cells lacking the functional enzyme are respiration-deficient but viable.

Another highly specialized import catalyst is the yeast protease that removes the sorting sequence from cytochrome *b*₂ (a protein imported from the cytoplasm) and the signal sequence from cytochrome oxidase subunit II (a protein synthesized inside the mitochondria) (refs 51–53; M. Behrens *et al.*, unpublished). The enzyme is termed 'inner-membrane protease I' because it is an integral inner-membrane protein exposed to the intermembrane space and because the inner membrane probably contains additional proteases participating in the maturation of imported precursors^{52,53}. The protease seems to consist of two or more non-identical subunits. One of them, of mass 21.4K, is similar in sequence to the *E. coli* leader peptidase and is encoded by the nuclear *IMP1* gene. Inactivation of *IMP1* is not lethal, but blocks maturation of cytochrome *b*₂ and renders the cells deficient in cytochrome oxidase and respiration (ref. 53; M. Behrens *et al.*, unpublished).

The inner-membrane protease I mediates the maturation, not the translocation, of precursors. With some of the other components, this distinction is not so clear-cut. For example, the MAS-protease is only required for translocation *in vivo*, but not in experiments with isolated mitochondria^{9,17,54}. This apparent discrepancy probably reflects a fundamental difference between the two import systems which is often ignored. Experiments with intact cells usually demand that the import machinery perform multiple rounds of catalysis; in contrast, most *in vitro* systems operate 'non-catalytically', because the import

TABLE 2 Components of mitochondrial protein import machinery in other organisms and their probable yeast homologues

Component	Location	Organism	Function	Refs	Yeast homologue
PEP	Matrix	<i>Neurospora</i>	Removal of matrix-targeting signal	10, 16	MAS1
MPP	Matrix	<i>Neurospora</i>	Removal of matrix-targeting signal	10, 16	MAS2
P55	Matrix	Rat	Removal of matrix-targeting signal	57	MAS2?
P52	Matrix	Rat	Removal of matrix-targeting signal	57	MAS1?
hsp60	Matrix	Maize	Assembly of mitochondrial protein products	58	hsp60
MOM72	Outer membrane	<i>Neurospora</i>	Import receptor	42	MAS70
MOM38	Outer membrane	<i>Neurospora</i>	Translocation across outer membrane?	32	ISP42
MOM19	Outer membrane	<i>Neurospora</i>	Import receptor	32, 46	MOM17?
29K protein	Outer membrane	Rat	Import receptor	45	p32?
Processing protease II	Matrix	Rat	?	55	?
Presequence binding protein	Cytosol	Rat liver	Antifolding	56	?

References for yeast proteins are listed in Table 1.

machinery is in large stoichiometric excess over the radiolabelled precursor protein. Experiments with isolated mitochondria may thus fail to uncover that a component transports precursors across the membranes.

Other organisms

Many of the import catalysts discussed here have also been discovered in other organisms, particularly in *N. crassa* (ref. 5). Others have been found in mammals or *Neurospora*, but not yet in yeast^{55,56}. Table 2 lists components found in systems other than yeast and attempts to define the equivalent yeast protein. Several of the components were tracked down by different approaches in different systems. Although each of these systems offers unique advantages, yeast will probably remain indispensable for demonstrating the physiological role of an import catalyst.

Future prospects

The picture that is emerging of mitochondrial import is one of a family of receptors on the outer membrane that feed into a central import pathway^{33,38}. The import receptors seem to aid import but are not essential *in vivo* or *in vitro*. These components are classified as functionally redundant. The essential components of the mitochondrial protein import machinery iden-

tified so far participate in the processing and folding of precursors and their transport across the outer membrane (Fig. 1). There are probably many essential components which are still unknown, including subunits of the protein transport channel across the inner membrane and other matrix proteins participating in the refolding and assembly of imported precursor proteins. Components essential in one organism may be functionally redundant in another. However, the facultative anaerobe *S. cerevisiae* tolerates alterations of its mitochondria that are lethal to many obligate aerobes; thus, if a mitochondrial component is essential for life in yeast, it should also be essential for obligate aerobes.

The formation of respiring mitochondria in yeast requires more than 100 nuclear genes whose inactivation prevents growth on a non-fermentable carbon source^{1,4}. Only five of these genes are known to be essential for viability, and they all encode proteins mediating the translocation and maturation of mitochondrial precursor proteins. This remarkable fact should be of considerable value in identifying additional import components. □

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Evidence for extreme mantle fractionation in early Archaean ultramafic rocks from northern Labrador

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Samarium–neodymium isotope data for tectonically interleaved fragments of lithospheric mantle and meta-komatiite from the North Atlantic craton provide the first direct record of mantle differentiation before 3,800 Myr ago. The results confirm the magnitude of light-rare-earth-element depletion in the early mantle, and also its depleted neodymium isotope composition. The mantle fragments were able to retain these ancient geochemical signatures by virtue of having been tectonically incorporated in buoyant felsic crust, thus escaping recycling and homogenization by mantle convection.

MODELS for the early chemical differentiation of the Earth depend strongly on inferences regarding primitive crust and mantle compositions, as well as knowledge of the extent and timescales of mantle fractionation^{1–4}. Unfortunately, such knowledge is limited by the relatively small database for early crustal compositions^{5–11}. The generally positive ϵ_{Nd} values (fractional deviation in parts per 10⁴ of the initial ¹⁴³Nd/¹⁴⁴Nd ratio from the chondritic ratio at that time) of between +1 and +5 exhibited by mantle-derived Archaean rocks require that their source regions had Sm/Nd ratios greater than the chondritic value (CHUR) for a significant period of time before melt formation and extraction¹². Geochemical fractionation in the early mantle, producing a reservoir with Sm/Nd greater than CHUR, is interpreted to have occurred by extraction of material enriched in light-rare-earth elements (LREEs) and with a low Sm/Nd ratio¹³. The nature of this enriched reservoir is uncertain; possibilities include older continental crust^{9,10}, enriched mantle⁹ or subducted mafic/ultramafic crust⁴. In view of the long half-life of ¹⁴⁷Sm (1.06 × 10¹¹ yr), generation of sufficient ¹⁴³Nd to produce measurable deviations of ¹⁴³Nd/¹⁴⁴Nd in the mantle requires that the enriched component escaped being recycled back into the upper mantle for a relatively long time (300–400 Myr). Positive initial ϵ_{Nd} values have been reported for pre-3,700-Myr Archaean rocks¹², indicating that these rocks were separated from a previously differentiated mantle, and that this depleted mantle reservoir must have formed before 4,100–4,200 Myr ago^{4,5,9}.

Here we report geochemical data for two suites of ultramafic rocks from the early Archaean gneiss complex in the Saglek–Hebron area of northern Labrador. One suite, comprising spinel-bearing meta-peridotites and meta-pyroxenites, is believed to be tectonically interleaved, ancient residual mantle. The other, dominantly pyroxenitic, suite represents a sequence of meta-komatiites associated with ~3,800-Myr-old supracrustal rocks. The two suites define Sm–Nd isochrons of 3,815 ± 121 Myr ($\epsilon_{\text{Nd}} = +2.97 \pm 0.60$) and 4,017 ± 194 Myr ($\epsilon_{\text{Nd}} = -2.11 \pm 2.33$) respectively. Although the isochron ages are identical within

error, the initial isotopic compositions are distinct. The positive ϵ_{Nd} value exhibited by the residual mantle suite provides the first direct confirmation of the extent of LREE depletion in early Archaean mantle. But as this suite is variably enriched in LREEs, the positive ϵ_{Nd} value at 3,800 Myr provides a minimum estimate of the extent of depletion in the contemporary mantle, implying that some pre-3,800-Myr mantle must have had an even higher Sm/Nd ratio, and hence a depleted Nd isotopic composition. These results support conclusions regarding the evolution of the depleted mantle based on Nd isotopic analyses of Archaean volcanic rocks and granitoids¹², and show that the mantle beneath the North Atlantic craton experienced significant geochemical differentiation before 3,800 Myr.

Geological Setting

Archaean gneisses in the Saglek–Hebron segment of the North Atlantic craton experienced a complex igneous and tectonothermal history between ~3,900 and 2,500 Myr (refs 6, 14–16). Ion microprobe (SHRIMP) data indicate that the plutonic protoliths of the early Archaean Uivak I gneisses formed between 3,700 and 3,800 Myr ago^{6,17}, and locally contain inherited xenocrystic components as old as 3,920 Myr (ref. 6). The Uivak I gneisses are interleaved with a heterogeneous group of supracrustal rocks termed the Nulliak assemblage¹⁸. Ion microprobe studies of Nulliak assemblage lithologies^{19,20} show that they contain zircons ranging in age from 3,780 to 3,850 Myr. The association of early Archaean gneisses in northern Labrador is similar in age and character to the Amitsôq gneisses and Akilia supracrustals in the Nuuk area of west Greenland²¹.

Ultramafic rocks are a subordinate, though distinct, component of the gneiss complex. They occur as tectonically emplaced units along structural contacts within ~3,700-Myr-old Uivak I gneisses, and as metavolcanic layers and dykes associated with Nulliak supracrustal sequences. The tectonically emplaced ultramafic units range in thickness from <2 to 300 m and form outcrops that can be traced up to 1–2 km along strike. Planar fabrics that result from ductile deformation are developed only weakly in marginal zones of ultramafic units, which are commonly dominated by narrow zones of serpentinite or biotite. The interiors of the ultramafic bodies are generally massive and exhibit compositional layering on scales of centimetres to metres, which are interpreted as a protolith feature. Lithologies include hercynite-bearing lherzolite, harzburgite, websterite, wehrlite and dunite. These mineral assemblages reflect either the original igneous mineralogy or the effects of high-grade metamorphism. Some units of pargasite-bearing websterite contain garnet surrounded by symplectic intergrowths of hercynitic spinel, anorthite-rich plagioclase and orthopyroxene. Such microstructures are interpreted as having been formed during tectonically induced decompression. The ultramafic bodies are distinct from cumulate ultramafic units associated with gabbroic intrusions within the gneiss complex²². In view of their structural setting, lithological association and mineral reactions, they are thought to be tectonically interleaved slices of early Archaean lithospheric residual mantle.

Nulliak supracrustal ultramafic units occur with amphibolites derived from mafic volcanic protoliths. They range from semi-continuous horizons, up to 2 km in strike length and 50–100 m wide, to small, highly attenuated, tectonically disrupted or agmatitic fragments ($<3 \times 0.25$ m) that occur within Uivak gneisses, or leucosome derived from Uivak gneiss protoliths. Outcrops of ultramafic and mafic rocks are commonly inter-layered on scales ranging from several centimetres to greater than a metre. Contacts between units range from sharp to diffuse. Primary igneous textures are not preserved owing to ductile deformation and to the effect of recrystallization under amphibolite to granulite facies conditions. The margins of bodies are commonly altered to monomineralic biotite or hornblende, as a result of metasomatic reaction with adjacent gneisses.

Major- and trace-element geochemistry

Samples were collected from several relatively large units in the vicinity of Iterungnek Fiord and Jerusalem Harbour, northeast of Hebron. Representative major- and trace-element compositions and petrographic details are given in Table 1. Although the samples exhibit varying degrees of secondary alteration which has clearly affected some elemental concentrations, particularly Ca, K, Rb, Sr and Pb, other elements seem to be affected relatively little either by metamorphism or by secondary alteration. The two ultramafic suites, which were initially subdivided using field criteria are discriminated clearly in Fig. 1. The meta-lherzolite, harzburgite and olivine websterite tectonic inclusions have Mg/Si and Al/Si ratios between 1.08–1.54 and 0.01–0.117 respectively. They exhibit a strong negative correlation on Fig. 1, and define a field similar to the 'mantle fractionation trend' in primitive peridotite nodules²³ and Alpine peridotites²⁴. Two samples (110C and 119A) have compositions close to that of the primitive mantle²³ and of model pyrolite²⁵. Geochemically less fertile samples, plotting to the left of these primitive compositions, are interpreted as refractory residues of earlier melt removal. A sample of meta-pyroxenite (106A) has a significantly lower Mg/Si and higher Al/Si ratio than other members of the suite and is interpreted as a liquid composition. The dominantly pyroxenitic suite associated with Nulliak supracrustal sequences have Mg/Si and Al/Si ratios that extend from values close to pyrolite and primitive mantle to compositions with significantly

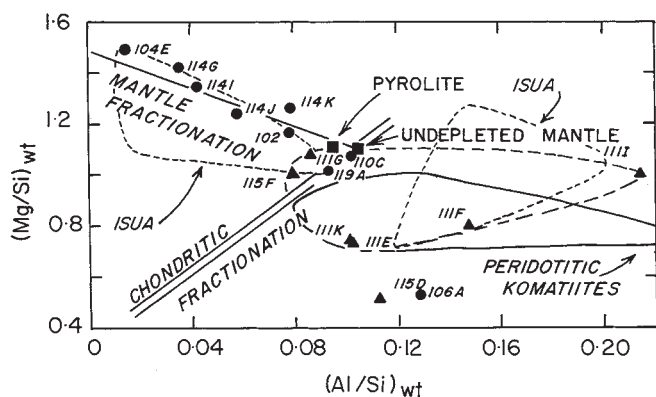


FIG. 1 Covariation between Mg/Si and Al/Si ratios. Meta-lherzolites, harzburgites and olivine websterites that occur as tectonic inclusions are shown as solid circles. They exhibit a strong negative correlation and occupy a field similar to the trajectory of the 'mantle fractionation' trend defined by primitive peridotite nodules²³ and Alpine peridotites²⁴. Sample 106A has a significantly lower Mg/Si and higher Al/Si ratio than other members of the suite and may represent a liquid composition. Meta-komatiites associated with Nulliak supracrustal sequences are shown as solid triangles. They exhibit Mg/Si and Al/Si ratios that extend from values close to pyrolite and primitive mantle, to compositions with significantly higher Al/Si ratios than the residual mantle suite. This field is similar to that for peridotitic komatiites from the Abitibi Belt²⁶. Also shown for comparison are data for early Archaean ultramafic rocks from Isua, West Greenland²⁷.

higher Al/Si ratios than the residual mantle suite (Fig. 1). This field is similar to that of peridotitic komatiites from the Abitibi Belt²⁶. In view of this similarity, and in spite of the absence of preserved quench textures, these ultramafic rocks are interpreted as meta-komatiites. Also shown for comparison in Fig. 1 are data for early Archaean ultramafic rocks from Isua, West Greenland²⁷.

Members of the residual mantle suite contain 36.9–47.5% MgO and 39.9–44.8% SiO₂, and 1,480–2,450 $\mu\text{g g}^{-1}$ Ni and 1,210–3,220 $\mu\text{g g}^{-1}$ Cr. The meta-clinopyroxenite (106A) associated with this suite has 22.6% MgO, 51.6% SiO₂ and 993 $\mu\text{g g}^{-1}$ Ni and 1,811 $\mu\text{g g}^{-1}$ Cr. Meta-komatiites have 19.3–38.3% MgO and 40.3–52.4% SiO₂, and 990–2,010 $\mu\text{g g}^{-1}$ Ni and 1,810–3,310 $\mu\text{g g}^{-1}$ Cr. The range in silica content indicates that compositions vary from peridotitic to basaltic komatiite. The high values of MgO, Ni and Cr shown by some meta-komatiites may be due to the presence of xenocrystic olivine in protoliths of these units.

Representative REE data for the two suites are shown in Fig. 2. Chondrite-normalized patterns for the residual mantle suite (tectonic inclusion) (Fig. 2a) are remarkably coherent and, except for the meta-harzburgite (104E), all show LREE enrichment to varying degrees. There is no clear correlation between degree of LREE enrichment and extent of serpentinization, and LREE abundances (in particular Ce) are not believed to have been influenced by hydrothermal alteration (compare refs 28 and 29). In addition, LREE enrichment also does not seem to be related to the proximity of the samples to felsic gneisses. The heavy REEs (HREEs) are not significantly fractionated (chondrite-normalized ratios $\text{Gd}_N/\text{Yb}_N = 0.80\text{--}1.03$), and most samples exhibit near-chondritic HREE abundances. These are somewhat lower than estimates of HREE abundances in the primitive mantle, which are 1.67 times the chondritic values³⁰. Shown for comparison in Fig. 2a are REE abundance patterns for primitive spinel lherzolite xenoliths³⁰. Except for enrichments in the elements La to Pr and the variable Eu anomalies shown by the Labrador suite, overall REE abundances are remarkably similar. The meta-pyroxenite (106A) has elevated levels of REEs (LREE abundances ~ 10 times chondritic) and $\text{La}_N/\text{Sm}_N = 1.48$, $\text{Ce}_N/\text{Yb}_N = 2.59$ and $\text{Gd}_N/\text{Yb}_N = 0.93$. These data, and the geochemical properties discussed previously for this sample (see above), support the interpretation that the pyroxenite represents a liquid composition. In view of the association of pyroxenites within the residual mantle suite, it is possible that the LREE enrichment shown by other members of the suite is related to the intrusion of liquids of similar composition. The meta-harzburgite (104E) has low concentrations of REEs ($0.1\text{--}0.2$ times chondritic) and $\text{La}_N/\text{Sm}_N = 0.72$, $\text{Ce}_N/\text{Yb}_N = 0.89$ and $\text{Gd}_N/\text{Yb}_N = 0.68$, indicating an extremely olivine-rich composition that does not seem to have been affected to the same degree by the LREE enrichment event as were other members of the suite. Most samples exhibit negative Eu anomalies ($\text{Eu}/\text{Eu}^* \approx 0.29\text{--}0.97$, where Eu^* is the interpolated Eu abundance assuming no Eu anomaly) that do not seem to be related to extent of serpentinization or metamorphic alteration.

In contrast to the tectonic ultramafic inclusions, the meta-komatiites with $>25\%$ MgO are generally depleted in LREEs, with LREEs $\sim 0.7\text{--}1.4$ times chondritic and HREEs $\sim 1.6\text{--}2.9$ times chondritic (Fig. 2b). Slight LREE enrichment (shown by $\text{La}_N/\text{Nd}_N = 0.97\text{--}1.90$) may reflect contamination by continental crust during eruption of the protoliths of the suite³² (see below). Except for La, Ce and Pr, the general shape of the REE patterns is similar to the field for peridotitic komatiites (Fig. 2b). The meta-komatiites also have variable Eu anomalies ($\text{Eu}/\text{Eu}^* \approx 0.65\text{--}1.66$) that do not seem to be related to extent of serpentinization. Also given in Table 1 are data for a specimen (115D) with higher content of REEs than the meta-peridotitic komatiites. This specimen is LREE-enriched and also displays a strong negative Eu anomaly ($\text{Eu}/\text{Eu}^* = 0.67$). In view of its geochemistry and field association, the protolith of this unit was

TABLE 1 Representative mineralogy and partial chemical analyses of early Archaean ultramafic rocks from Labrador

	Residual mantle suite						Meta-komatiite suite					
KC-87/-	104E	114G	114I	114K	119A	106A	111G	111K	111E	111F	111I	115D
Si	19.4	19.9	19.9	20.1	21.0	25.6	21.2	23.3	23.8	21.7	19.9	23.7
Mg	28.9	28.2	27.4	25.3	21.4	15.4	22.9	17.3	17.4	17.2	19.9	12.4
Al	0.28	0.72	0.85	1.59	1.97	2.12	1.84	2.39	2.46	3.21	4.25	2.67
TiO ₂	0.01	0.07	0.10	0.24	0.18	0.13	0.16	0.17	0.16	0.25	0.36	0.46
Mg#*	0.80	0.81	0.82	0.81	0.75	0.78	0.76	0.76	0.70	0.65	0.73	0.61
Cr	1749	1254	1489	1206	2740	1811	2536	2280	3081	3313	2624	2418
Ni	2447	2395	2390	2384	1783	993	2005	1279	1514	1307	1194	1060
La	0.06	0.52	0.65	0.88	0.71	3.00	0.32	0.41	0.53	0.51	0.40	2.71
Ce	0.17	1.15	1.42	1.97	1.56	10.1	0.71	0.92	1.08	1.14	0.96	7.19
Pr	0.03	0.15	0.18	0.28	0.21	1.50	0.12	0.14	0.15	0.15	0.16	1.09
Nd	0.14	0.64	0.75	1.18	1.03	5.74	0.57	0.75	0.66	0.52	0.84	4.95
Sm	0.05	0.20	0.22	0.29	0.32	1.27	0.26	0.35	0.26	0.23	0.51	1.42
Eu	0.004	0.03	0.03	0.10	0.09	0.32	0.11	0.23	0.07	0.08	0.15	0.33
Gd	0.04	0.20	0.23	0.34	0.43	1.17	0.40	0.51	0.41	0.49	0.77	1.56
Ho	0.01	0.05	0.07	0.09	0.14	0.31	0.13	0.18	0.19	0.21	0.25	0.34
Yb	0.05	0.20	0.24	0.27	0.41	1.01	0.40	0.57	0.65	0.60	0.71	0.84
Y	0.34	1.67	1.73	2.15	3.18	7.84	2.96	4.06	4.65	4.94	5.34	6.81
Zr	0.63	5.20	4.26	11.4	7.71	12.9	5.75	7.24	5.69	6.37	11.2	26.5
Lu	0.01	0.03	0.04	0.04	0.07	0.15	0.08	0.09	0.10	0.09	0.11	0.13
Hf	0.03	0.17	0.14	0.37	0.26	0.32	0.20	0.25	0.18	0.33	0.44	0.92
Nb	0.53	0.52	0.36	0.40	1.20	0.54	0.32	0.44	0.48	0.25	0.28	1.38
Th	0.03	0.04	0.10	0.16	0.27	0.16	0.10	0.06	0.20	0.03	0.04	0.35
Y/Ho	34.0	33.4	24.7	23.9	22.7	25.2	22.7	22.5	24.5	23.5	21.4	20.0
Nb/Th	17.7	13.0	3.60	2.50	4.44	3.60	2.91	7.33	2.40	8.33	7.00	3.94
Zr/Hf	21.0	31.6	30.4	30.8	29.7	40.3	28.8	29.0	31.6	19.3	25.5	28.8
Hf/Lu	3.00	5.67	3.50	9.23	3.71	2.13	2.50	2.78	1.80	3.67	4.00	7.08
ol	10	5	20	40	45		28	10	5	10	20	10
opx	13	15	25	25	23	5	30	35	50	50	40	30
cpx	5		15	32	20	35	10	25	15		27	20
amph						42	30	15	30	30	5	30
spinel	2			3	2	3	2			10	8	10
serp	70	80	40		10							
phlog						10		15				
carb						5						

Data for Si, Mg and Al, and titania are quoted as wt% (anhydrous). These, together with Cr and Ni ($\mu\text{g g}^{-1}$) were determined by X-ray fluorescence. Remaining trace-element data (expressed as $\mu\text{g g}^{-1}$) were determined by ICPMS (inductively coupled plasma mass spectrometry) at Memorial University of Newfoundland and Lawrence Livermore National Laboratories. External precision of the ICPMS data (except Th and Gd) is 3–6% (1 σ). Precision of Th and Gd is 10% (1 σ). Specimen details: KC-87-104E, meta-harzburgerite; KC-87-114G, meta-harzburgerite; KC-87-114I, meta-lherzolite; KC-87-114K, meta-olivine websterite; KC-87-119A, meta-lherzolite; KC-87-106A, meta-pyroxenite; KC-87-111, series was collected from single large unit of Nulliak Assemblage meta-pyroxenite and meta-peridotite near Jerusalem Harbour. Full chemical analyses are available from K.D.C.

* Mg# = Mg/(Mg + Fe).

probably a meta-basaltic komatiite.

From the coherence of the patterns shown in Fig. 2, it seems that the REEs have not been disturbed significantly during metamorphism. Immobility of the HREEs and Y is also shown by the limited range in Y/Ho ratios exhibited by the residual mantle and meta-komatiite suites (22.7–34.0 and 20.0–24.5 respectively), and by the fact that these values are close to the primitive mantle ratio³¹ of 28. The high-field-strength elements (HFSEs) seem also to have been little disturbed by alteration. Zr/Hf ratios (Table 1) are close to the primitive-mantle value³¹ of 37. Nb/Th ratios range from 2.5 to 17.7 and from 2.9 to 8.33 in the residual mantle and meta-komatiite suites respectively (compare the primitive-mantle ratio³³ of 8). The range in Nb/Th of the residual mantle suite, from 17.7 in a refractory dunite to between 13 and 2.5, reflects either variable mantle enrichment or crustal contamination. Archaean komatiites display a range of Nb/Th ratios³³ of 7–15. Komatiites contaminated by continental crust, however, may have Nb/Th ratios <1 (ref. 33). Low Nb/Th ratios exhibited by some Nulliak meta-komatiites may therefore reflect crustal contamination.

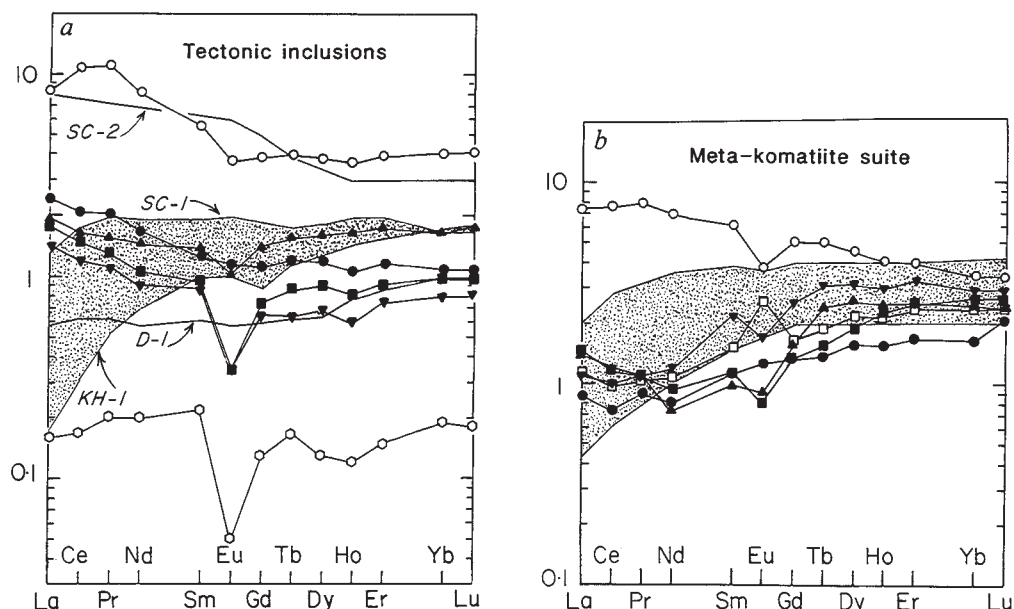
A number of workers^{34,35} have suggested that Archaean komatiites show significant chemical variation with time, with early Archaean (~3,500–3,800-Myr-old) komatiites having lower Al₂O₃/TiO₂ and higher CaO/Al₂O₃ and Gd_N/Yb_N ratios than later Archaean (~2,700–2,900-Myr-old) komatiites. These differences have been ascribed to removal of garnet during

petrogenesis of the older komatiites. Existing data for early Archaean komatiites are, however, drawn mainly from the Barberton greenstone belt. The early Archaean Nulliak meta-komatiites and members of the residual mantle suite in Labrador have Gd_N/Yb_N ratios consistently <1, CaO/Al₂O₃ ratios less than or equal to 1, and Al₂O₃/TiO₂ ratios greater than 10. This suggests that these units may have been derived from a mantle source that was enriched in majorite³⁴. These data indicate that models for Archaean mantle evolution³⁴ based on the temporal evolution of komatiite chemistry are not supported by the results for ultramafic rocks older than 3,500 Myr.

Sm–Nd isotope systematics

Neodymium isotope data for the two suites are given in Table 2 and plotted in Fig. 3. ¹⁴⁷Sm/¹⁴⁴Nd ratios for five members of the residual mantle suite range from 0.14 to 0.20 and are extremely well correlated with ¹⁴³Nd/¹⁴⁴Nd. Measured $\epsilon_{\text{Nd}}(0)$ (when $t=0$ corresponds to the present day) values range from +5.36 to –24.21. The data define a Model 1 isochron³⁶ (mean-square weighted deviation MSWD = 0.3) with a slope equivalent to an age of $3,815 \pm 121$ Myr and an initial ¹⁴³Nd/¹⁴⁴Nd ratio of 0.50782 ± 3 ($\epsilon_{\text{Nd}}(t) = +2.97 \pm 0.60$). Uncertainties in $\epsilon_{\text{Nd}}(t)$ values were calculated following the method of Fletcher and Rosman³⁷. Ultramafic members of the meta-komatiite suite range in ¹⁴⁷Sm/¹⁴⁴Nd ratios from 0.23 to 0.31. Present-day $\epsilon_{\text{Nd}}(0)$ values for the suite vary from +16.4 to +56.4 and reflect the

FIG. 2 Chondrite-normalized REE diagrams showing data for the two suites of ultramafic lithologies. *a*, LREE-enriched suite (tectonic inclusions). Open circles: KC-87-106A; solid triangles: KC-87-119A; solid circles: KC-87-114K; solid squares: KC-87-114I; inverted triangles: KC-87-114G; open hexagons: KC-87-104E. Shown for comparison are REE patterns for spinel-bearing peridotites (SC-1: San Carlos; KH-1: Kilbourne Hole; D-1: Dreiser Weiher) from Jochum *et al.*³¹ and for a group 2 San Carlos inclusion⁵¹ (SC-2). *b*, LREE-depleted meta-komatiite suite. Open circles: KC-87-115D; solid circles: KC-87-111G; open squares: KC-87-111K; inverted solid triangles: KC-87-111I; solid triangles: KC-87-111F; solid squares: KC-87-111E. The stippled area in these figures is the field for peridotitic komatiites^{52,53}.



time-integrated effect of extreme depletion in Nd ($f_{\text{Sm}/\text{Nd}} = +0.16$ to $+0.59$). The isotope data are less well correlated than the data for the 'residual mantle' suite, and yield a Model 2 isochron equivalent to an age of $3,825 \pm 168$ Myr (MSWD = 13.7) with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50767 ± 8 ($\epsilon_{\text{Nd}}(t) = +0.23 \pm 1.58$). When regressed with data for the meta-basaltic komatiite, the samples show a greater dispersion in $^{147}\text{Sm}/^{144}\text{Nd}$ (0.18–0.31) and yield a Model 3 isochron³⁶ with slope equivalent to an age of $4,017 \pm 194$ Myr (MSWD = 24) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50730 ± 11 ($\epsilon_{\text{Nd}}(t) = -2.11 \pm 2.33$). This age and $\epsilon_{\text{Nd}}(t)$ are the same, within error, as results obtained by excluding the meta-basaltic komatiite samples.

Although the ages of the two suites are the same within error, the $\epsilon_{\text{Nd}}(t)$ values are statistically different at the 2σ level. If one can assume that the ultramafic rocks defining the $\sim 3,800$ -Myr age are cogenetic, then the $\epsilon_{\text{Nd}}(t)$ value of $+2.97 \pm 0.60$ exhibited by this suite indicates that contemporary 3,800-Myr-old mantle was depleted in LREEs, corresponding to an $f_{\text{Sm}/\text{Nd}}$ value of $+0.16$. This supports the conclusions of previous studies that early depleted mantle exists beneath the North Atlantic craton^{5,6,9}. The negative $\epsilon_{\text{Nd}}(t)$ of -2.11 ± 2.33 displayed by the composite komatiitic Nulliak suite could also be significant. If the correlation defined by the composite suite is an isochron,

then the $\epsilon_{\text{Nd}}(t)$ value could be interpreted as indicating that the suite was derived from melting of an enriched mantle source with a low Sm/Nd ratio relative to CHUR, yielding an $f_{\text{Sm}/\text{Nd}}$ value of -0.15 . However, in view of the low abundances of REEs, strong LREE depletion (Fig. 2*b*) and extreme fractionation in Sm/Nd ratios exhibited by the meta-peridotitic komatiites (Table 2), it is unlikely that these geochemical signatures could be generated or inherited from such a mantle source by the high degrees (30–50%) of partial melting required to account for the genesis of peridotitic komatiites^{26,38}. If, on the other hand, enriched and depleted reservoirs were formed contemporaneously within the mantle, the linear correlation may be interpreted as a mantle isochron.

An alternative explanation is that the correlation between $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{147}\text{Sm}/^{144}\text{Nd}$ ratios shown in Fig. 3 is the result of interaction between the protoliths of the meta-komatiite suite and a LREE-enriched component. This interpretation is supported by the LREE data for the suite. The LREE-enriched component could be pre-existing crust or an enriched layer in the mantle. Contamination of LREE-depleted komatiitic liquid by crust could occur by thermally induced erosion³². Well correlated $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{147}\text{Sm}/^{144}\text{Nd}$ mixing lines that give equivocal age information are commonly obtained for rocks affected by such a process³⁹.

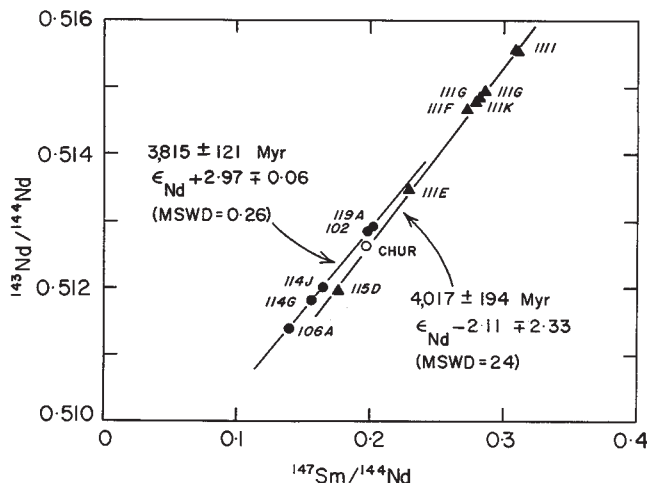
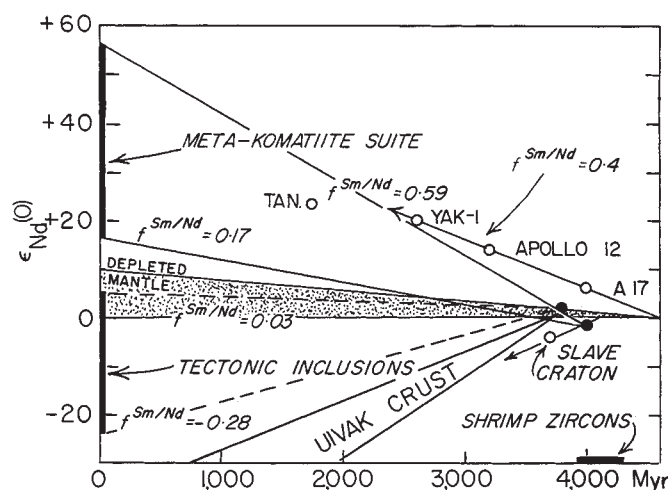


FIG. 3 Sm-Nd evolution diagram for the two suites of ultramafics. The residual mantle suite (solid circles) yields an age of $3,815 \pm 121$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50782 ± 3 ($\epsilon_{\text{Nd}}(t) = +2.97 \pm 0.60$). Ultramafic members of the meta-komatiite suite (solid triangles) exhibit an extreme range in $^{147}\text{Sm}/^{144}\text{Nd}$ ratios yielding present-day $\epsilon_{\text{Nd}}(0)$ values that vary from $+16.4$ to $+56.44$. These values reflect the time-integrated effect of extreme depletion in Nd ($f_{\text{Sm}/\text{Nd}} = +0.16$ to $+0.59$). When combined with isotope data for the meta-basaltic komatiite, the total population yields an isochron with slope equivalent to an age of $4,017 \pm 194$ Myr (MSWD = 24) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50730 ± 11 ($\epsilon_{\text{Nd}}(t) = -2.11 \pm 2.33$).

FIG. 4 Evolution vectors corresponding to $f_{\text{Sm/Nd}} = +0.16$ to $+0.59$ for the meta-komatiite suite. Although interpretation of the initial isotopic composition is equivocal, the data clearly indicate that these rocks represent partial melts of an extremely depleted source with a Sm/Nd ratio substantially greater than that commonly assumed for the depleted mantle ($f_{\text{Sm/Nd}} = 0.09$). The stippled field represents compositions of melts derived from the depleted mantle. The $\epsilon_{\text{Nd}}(t)$ value of $+2.97 \pm 0.60$ exhibited by the LREE-enriched tectonic inclusions indicates that contemporary 3,800-Myr-old mantle was depleted in LREEs. For comparison, data are also shown for ancient eclogites in the sub-continental lithosphere (YAK-1 ($\epsilon_{\text{Nd}} = +20$ at 2,600 Myr) from Siberia⁴² and TAN ($\epsilon_{\text{Nd}} = +23.4$ at 1,750 Myr) from Tanzania⁴³) and for pre-3,800-Myr Apollo 12 and 17 lunar basalts⁴⁴. Also shown are evolution vectors for the $\sim 3,950$ -Myr-old Acasta gneisses from the Slave Craton^{10,11} and the $\sim 3,700$ -Myr-old Uivak gneisses⁶.



Irrespective of the significance of the meta-komatiite 'isochron', it yields the same age for the supracrustal association, within error, as that based on SHRIMP zircon data^{19,20}. Values of $\epsilon_{\text{Nd}}(t=3,800 \text{ Myr})$ for the meta-komatiites are distinctly more negative and heterogeneous than those exhibited by the residual mantle suite (Table 2). If these negative ϵ_{Nd} values for meta-komatiites belonging to the Nulliak suite are the result of interaction with pre-existing crust, what was the character of this crust and what was the nature of the interaction? Bowring *et al.*^{10,11} have recently reported 3,960-Myr-old gneisses in the Northwest Territories, Canada, that are enriched in LREEs relative to CHUR ($\epsilon_{\text{Nd}}(t) = -2$). SHRIMP geochronological studies of zircons from Uivak gneisses⁶ and Nulliak felsic quartzites¹⁹ from Labrador, and of detrital zircons from Australia^{40,41}, have also been used to infer the existence of pre-3,850-Myr crust. In addition, variable positive ϵ_{Nd} values for $\sim 3,800$ -Myr-old metasedimentary rocks from Isua, West Greenland, have been used⁹ to argue for the existence of a complementary enriched reservoir. Although the existence of ancient (pre-3,800-Myr) terrestrial crust seems inescapable, we do not yet know the volume of this reservoir and specifically whether this volume

is sufficient for the reservoir to represent the complement of the early Archaean depleted mantle⁴.

Implications for mantle evolution

As komatiites are commonly believed to be formed by extensive partial melting of the mantle³⁸, the extremely depleted character of this suite suggests that the early Archaean mantle was strongly depleted in LREEs. Evolutionary paths that yield $f_{\text{Sm/Nd}} = +0.16$ to $+0.59$ (the extreme range of Sm/Nd ratios exhibited by the meta-komatiite suite) are shown in Fig. 4. Although there is no unambiguous interpretation of the initial isotopic composition, the data clearly indicate that these rocks represent partial melts of an extremely depleted source with an Sm/Nd ratio substantially greater than that commonly assumed for the depleted mantle ($f_{\text{Sm/Nd}} = 0.09$). Similar conclusions regarding the existence in the sub-continental lithosphere of reservoirs with $\epsilon_{\text{Nd}}(t) = +20$ for $t = 2,600 \text{ Myr}$ and $\epsilon_{\text{Nd}}(t) = +23.4$ for 1,750 Myr have been drawn by McCulloch *et al.*⁴² and Jagoutz⁴³ using data for eclogite inclusions in kimberlite. These extremely radiogenic ϵ_{Nd} isotopic compositions are thought to reflect crystal/liquid differentiation in protoliths of the eclogites

TABLE 2 Sm-Nd isotope data for early Archaean ultramafic rocks from Labrador

Sample	Sm ($\mu\text{g g}^{-1}$)	Nd ($\mu\text{g g}^{-1}$)	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$\epsilon_{\text{Nd}}(0)$	$\epsilon_{\text{Nd}}(3.8 \text{ Gyr})$	$f_{\text{Sm/Nd}}$
Residual mantle suite							
KC-87-119A	0.39	1.182	0.2017	0.512913 ± 14	+5.36	+2.97	+0.0259
KC-87-102	0.41	1.241	0.1984	0.512840 ± 10	+3.94	+3.15	+0.0094
KC-87-114J	0.23	0.835	0.1656	0.512010 ± 16	-12.26	+2.85	-0.1575
KC-87-114G	0.21	0.819	0.1578	0.511817 ± 24	-16.01	+2.87	-0.1972
KC-87-106A	1.47	6.267	0.1414	0.511397 ± 7	-24.21	+2.63	-0.2808
Meta-komatiite suite							
KC-87-111I*	0.45	0.889	0.3084	0.515531 ± 77	+56.43	+2.30	+0.5689
KC-87-111I	0.46	0.885	0.3129	0.515521 ± 18	+56.24	-0.10	+0.5916
KC-87-111G	0.30	0.629	0.2868	0.514934 ± 29	+44.79	+1.12	+0.4590
KC-87-111G*	0.30	0.636	0.2845	0.514811 ± 15	+42.39	-0.18	+0.4472
KC-87-111K	0.39	0.829	0.2828	0.514821 ± 14	+42.57	+0.85	+0.4382
KC-87-111K*	0.39	0.830	0.2814	0.514811 ± 18	+42.39	+1.34	+0.4315
KC-87-111F	0.30	0.670	0.2742	0.514659 ± 18	+39.42	+1.87	+0.3946
KC-87-111E	0.27	0.700	0.2297	0.513477 ± 12	+16.37	+0.37	+0.1681
Meta-basaltic komatiite							
KC-87-115D	1.52	5.181	0.1774	0.511949 ± 31	-13.43	-4.12	-0.0978

Sm and Nd were separated by cation-exchange techniques from a mixture of homogeneous rock powder that had been spiked with a ^{149}Sm - ^{150}Nd spike and dissolved in an acid digestion bomb. Total processing blanks for Nd and Sm are $\sim 30 \text{ pg}$ per dissolution. Isotope analyses were carried out on VG 354 Sector mass spectrometers at University of California at Santa Cruz and VG Isotech. Neodymium isotope ratios were normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$ to correct for mass fractionation. Analyses were carried out using static multi-collection techniques. Samples were loaded with 0.01 M phosphoric acid onto Ta side filaments of a triple filament bead assembly with a Re ionizing filament. They were analyzed as the metal-ion with a $1 \times 10^{-12} \text{ A}$ aiming intensity. Analyses of neodymium at La Jolla made during the course of this study yielded $^{143}\text{Nd}/^{144}\text{Nd} = 0.511865$ relative standard deviation = 30 p.p.m. (1σ) at both VG Isotech and UCSC. Duplicate analyses are indicated by *. Reported errors are 2 standard errors of the mean.

as well as subsolidus fractionation of Sm/Nd between garnet and clinopyroxene. These eclogite data and initial ϵ_{Nd} isotopic compositions for pre-3,800-Myr lunar basalts⁴⁴, thought to have been derived from sources that experienced similar extreme fractionation in Sm/Nd, are shown for comparison in Fig. 4. Preservation of such a refractory depleted-mantle reservoir by processes associated with the formation of early continental crust is unlikely, unless huge volumes of crust were extracted and prevented from being recycled back into the mantle. This scenario is implausible, given the obviously metastable character of the proto-crust and the extreme rarity of rocks older than 3,900 Myr in the geological record. In fact, Chase and Patchett⁴ have argued that it is difficult to explain the generation even of ϵ_{Nd} values in the range +3 to +5 by 3,500 Myr ago in terms of a model that involved solely formation of continental crust. To accommodate these difficulties, they modelled the generation of an enriched reservoir by subduction and isolation in the mantle of early mafic 'oceanic crust' over timescales >400 Myr. Based on estimates⁴⁵ of the degassing rate of water, however, it is unlikely that a significant hydrosphere had formed before 4,000 Myr ago. As a result, there may have been little opportunity for the generation of large amounts of hydrothermally altered mafic crust. This would also explain why examples of pre-3,800-Myr sialic crust are rare⁴⁶.

There has been considerable speculation concerning the role of a magma ocean during the early evolution of the mantle⁴⁷⁻⁴⁹.

In particular, crystallization of this ocean would result in the formation of garnet-rich olivine-clinopyroxene-orthopyroxene cumulates at relatively shallow mantle depths (<400 km) and majorite-garnet-enriched cumulates at depths greater than 400 km. Based on current estimates of crystal/liquid distribution coefficients^{34,50}, such processes would yield HREE-enriched cumulates, with Sm/Nd ratios greater than chondritic (assuming a chondritic source), $\text{Gd}_\text{N}/\text{Yb}_\text{N} < 1$ and $\text{Al}_2\text{O}_3/\text{TiO}_2$ ratios generally greater than 20. Decay of ^{147}Sm in such a reservoir on timescales of >300 Myr should yield ϵ_{Nd} values >1. Similar geochemical and isotopic characteristics are exhibited by the Labrador ultramafics, strongly suggesting that a garnet-enriched source was involved in their genesis. Our results therefore suggest that extensive chemical differentiation of the Earth occurred over 4,000 Myr ago. These geochemical signatures are preserved in these early Archaean ultramafic rocks because they have been tectonically isolated in units of extremely ancient crust, and have thereby escaped being recycled into the mantle. The presence of such a LREE-depleted reservoir in the early Earth also provides insight into the significance of the initial $\epsilon_{\text{Nd}}(t)$ value of the residual mantle suite. This suite has been shown to have been variably enriched in LREEs, possibly by introduction of melts enriched in incompatible elements earlier than 3,800 Myr ago. If this model is correct, it implies that the $\epsilon_{\text{Nd}}(t)$ value of +3 at 3,800 Myr provides a minimum estimate of the extent of LREE depletion in pre-3,800-Myr mantle. □

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A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein β -adaptin

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Four high-molecular-weight proteins form the main subunits of the coat of Golgi-derived (non-clathrin) coated vesicles. One of these coat proteins, β -COP, is identical to a Golgi-associated protein of relative mass 110,000 (110K) that shares homology with the adaptin proteins of clathrin-coated vesicles. This connection, and the comparable molecular weights of the coat proteins of Golgi-derived and clathrin-coated vesicles, indicates that they may be structurally related. The identification of β -COP as the 110K protein explains the blocking of secretion by the drug brefeldin A.

INTRACELLULAR traffic between the membrane compartments of eukaryotic cells relies on the movement of vesicular carriers¹⁻⁴. Clathrin-coated vesicles⁵ mediate selective transport events⁶. Vesicular carriers within the Golgi apparatus, however, form with a coat distinct from that of clathrin-coated vesicles⁷⁻⁹ and act as non-selective 'bulk flow' carriers of the constitutive biosynthetic pathway⁹⁻¹¹. Although many of the constituents of the clathrin coat have been well characterized^{12,13}, nothing is known about the proteins of the non-clathrin coat.

Golgi-derived (non-clathrin) coated vesicles can be produced *in vitro* in a cell-free system that reconstitutes intercompartmental protein transport¹⁴⁻¹⁶: incubation with cytosol and ATP causes a population of non-clathrin-coated vesicles and buds to form *de novo* from the previously uncoated, flattened cisternae^{9,17,18}. The non-hydrolysable nucleotide analogue GTP- γ -S inhibits uncoating in this reaction, causing these coated vesicles to accumulate by ~5-fold¹⁹. These Golgi-derived coated vesicles can be purified from such GTP- γ -S-containing reactions²⁰.

We report here an improved purification that has enabled us to identify four stoichiometric subunits of the coat, the coat proteins (COPs): α -COP (160K), β -COP (110K), γ -COP (98K), and δ -COP (61K). Microsequencing of β -COP reveals that it is identical to a Golgi-associated peripheral membrane protein of unknown function but identified by a monoclonal antibody raised against microtubule-associated proteins (MAPs; ref. 21). The predicted amino-acid sequence of β -COP is homologous to that of the β -adaptin proteins of clathrin-coated vesicles (R. Duden, G. Griffiths, R. Frank, P. Argos and T. E. Kreis, manuscript submitted), indicating that the two types of coated vesicles

must be similar. This same 110K protein, which we have now identified as a coat subunit, dissociates rapidly from the Golgi apparatus *in vivo* upon addition of brefeldin A²², a drug that blocks secretion^{23,24}. This suggests that assembly of non-clathrin-coated vesicles is a likely target for brefeldin A.

Purification of Golgi-derived vesicles

Using rabbit liver Golgi as a membrane source and cytosol from bovine brain, we have noted that proteins of molecular weights 160K and 105-95K were the most likely candidates for vesicle-specific proteins in a highly purified preparation of non-clathrin-coated vesicles²⁰, but they were neither the only, nor even the major proteins present. We could not therefore easily sequence the principal vesicle-specific proteins, nor could we definitively identify any proteins as vesicle constituents.

Considering that the 160 K and 105-95K proteins were most probably vesicle-specific and that the vesicles had a buoyant density of ~1.18 g ml⁻¹, we modified our purification procedure to maximize the purity and yield of these vesicle markers at that density. The chief revisions were (1) use of Golgi membranes from Chinese hamster ovary (CHO) cells instead of rabbit liver; (2) elimination of a sucrose cushion in the initial membrane collection; (3) inclusion of a low-salt wash before the high-salt wash to release contaminating proteins while still retaining coated vesicles attached to cisternae; (4) no pelleting of the coated vesicles once they had been stripped from the cisternae; and (5) use of a steeper isopycnic gradient into which the vesicles were centrifuged from the top (rather than floated up from below).

Four major proteins band very tightly on the isopycnic gradient in fractions 7-9 (Fig. 1a) at ~40.5% sucrose (1.18 g ml⁻¹; Fig. 1b), the density expected for the non-clathrin-coated vesicles: proteins of 160K, 110K and 98K (previously described as 160K and 105-95K), and 61K are the main proteins of the vesicle-containing fractions. (Other vesicle-specific proteins of lower molecular weight are present²⁵, including GTP-binding proteins (T.S. and J.E.R., manuscript in preparation).) In incubations without GTP- γ -S, these four proteins are not evident in the density gradient (data not shown). Other proteins in the vesicle-containing fractions are contaminants, peaking at a different density from the coated vesicles.

If these four proteins are coat proteins rather than cargo, they should also be present in coated vesicles derived from another membrane source, for example, rabbit liver Golgi, and indeed this is the case. The protein composition of the top portion of the isopycnic gradient differs between preparations from CHO Golgi and rabbit liver Golgi (compare fractions 13-17 in Fig. 1a and c, respectively), but the four proteins specific to CHO Golgi-derived coated vesicles are also the major proteins of rabbit liver Golgi-derived vesicles, also present in fractions 7-9

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(Fig. 1c) at ~41.5% sucrose (1.18 g ml^{-1} ; Fig. 1d). There are differences between Golgi-derived vesicles from CHO and rabbit liver: the 98K protein of coated vesicles from rabbit liver Golgi can, under certain electrophoretic conditions, split into two bands (data not shown), and the 110K protein migrates as a broad band—perhaps a minor protein with a similar molecular weight is also present. There are other minor proteins that peak with the four major proteins; several are common to coated vesicles from both membrane sources and so could represent other vesicle-associated proteins that are part of the transport machinery.

Identification of coat proteins

The four major proteins in purified Golgi coated vesicles are coat proteins because they are membrane-associated and externally disposed. Thus, when coated vesicles are produced from Golgi membranes from vesicular stomatitis virus (VSV)-infected

CHO cells (as in Fig. 1a and b), a peak of VSV-G protein (a transported integral membrane protein), coincides on the gradient with the peak of the vesicle-specific proteins (fractions 7–9; Fig. 2), as would be expected if these vesicle-specific proteins are associated with transport vesicles. Furthermore, if a peak vesicle fraction is adjusted to 50% sucrose, layered underneath a sucrose gradient and centrifuged, these four proteins float up to roughly the density at which they were found originally (data not shown), confirming that these four proteins are membrane-associated.

The four proteins are located on the external surface of vesicles. All are sensitive to added protease (compare lanes 1 and 2 in Fig. 3a), whereas albumin, a secretory protein of liver found within the vesicle lumen when the vesicles have been prepared from rabbit liver Golgi, remains protected by the membrane under these conditions (compare lanes A and B in Fig. 3b). The albumin, however, is sensitive to added protease

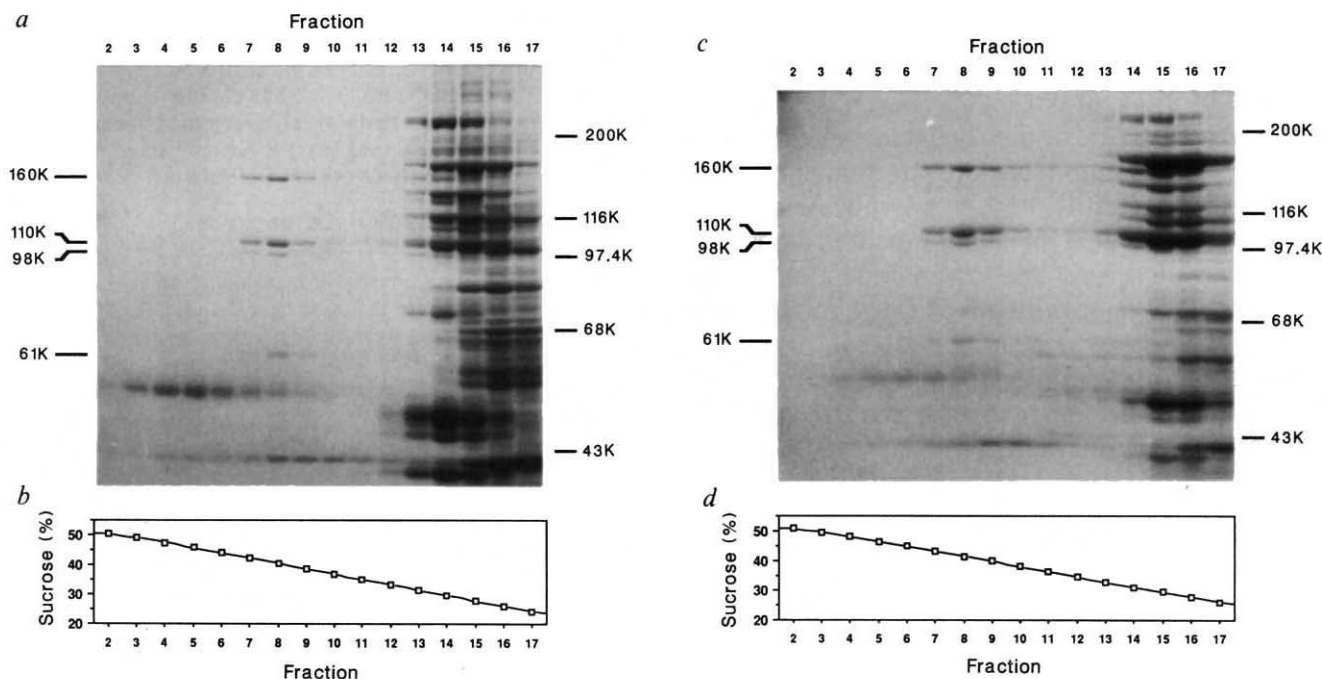


FIG. 1 Protein composition of Golgi-derived non-clathrin-coated vesicles. *a*, Coomassie brilliant blue (CBB)-stained SDS-PAGE gel (7.5% acrylamide) showing fractions 2–17 of the isopycnic gradient from a coated vesicle preparation using CHO Golgi. The four major vesicle-specific proteins are indicated on the left by their molecular weights estimated from SDS-PAGE. Molecular weight standards are indicated on the right. *b*, Per cent sucrose (w/w) composition of the fractions in *a* as determined by refractometry. *c*, CBB-stained SDS-PAGE gel showing fractions from a coated vesicle preparation using rabbit liver Golgi (proteins indicated as in *a*). *d*, Per cent sucrose composition of the fractions in *c*.

METHODS. Reaction mix for CHO membranes: A 60-ml reaction mixture was assembled on ice consisting of 20 mM KCl (total after addition of cytosol), 25 mM HEPES-KOH (pH 7.0), 2.5 mM magnesium acetate, 0.2 M sucrose (total after addition of membranes), 125 μM DTT, 50 μM ATP, 250 μM UTP, 5 mM creatine phosphate, 8.0 IU ml^{-1} creatine kinase, 25 $\mu\text{g ml}^{-1}$ RNase A, and 2.4 mg ml^{-1} bovine brain cytosol (prepared as in ref. 20). To this assembled mixture was added GTP- γ -S (Boehringer Mannheim) to 20 μM , and CHO Golgi membranes (from VSV-infected 15B cells^{14,15}) to 50–100 $\mu\text{g ml}^{-1}$, with the GTP- γ -S (120 μl) and membranes (6 ml) being mixed together before addition. Reaction mix for rabbit liver membranes: A 30-ml reaction mixture was assembled identical to the one above except for the following: The reaction mixture was assembled directly into the glass centrifuge tube used subsequently (see below), DTT was 250 μM , bovine brain cytosol was 4.8 mg ml^{-1} , and rabbit liver Golgi membranes (ref. 20) were added to 50–120 $\mu\text{g ml}^{-1}$. Purification procedure: The complete reaction mixture was immediately pipetted into two 30-ml Corex centrifuge tubes and incubated for 15 min at 37 °C; after this incubation the tubes were chilled for 10 min in an ice/water bath (all subsequent steps were carried

out on ice), and the membranes were collected by centrifugation for 30 min at 10,500 r.p.m. in a Sorvall SA-600 rotor ($g_{av}=11,400g$; k (clearing factor) $\approx 1,200$). The reaction mixture was removed by aspiration, and the membrane pellet resuspended in a total of 0.6 ml of low-salt stripping buffer (50 mM KCl/25 mM HEPES-KOH (pH 7.2)/2.5 mM magnesium acetate/0.2 M sucrose) using a Pipetman P-1000. Resuspended membranes were incubated on ice for 15 min and then collected by microcentrifugation for 10 min. Supernatant was removed by aspiration and discarded, and the membrane pellet resuspended as before into 0.6 ml high-salt stripping buffer (as low-salt buffer, except 250 mM in KCl). After incubation for 15 min on ice, the larger contaminating membranes were removed by microcentrifugation as before. The supernatant (600 μl) was recovered, transferred to a new tube, and microcentrifuged for 15 min. Only the top 545 μl of supernatant was recovered ('crude' coated vesicles), placed into a new tube, and adjusted to 20% (w/w) sucrose by the addition of 55% stripping buffer (SB; same as high-salt buffer, but at the indicated sucrose concentration (w/w)). The crude vesicles were layered on an initially discontinuous sucrose gradient formed by layering 714 μl of each of the following in an SW55 tube: 50% SB, 45% SB, 40% SB, 35% SB, 30% SB, and 25% SB. The gradient was centrifuged for 18 h at 32,500 r.p.m. in a Beckman SW55 rotor ($g_{av}=100,000g$) and fractionated from the bottom using a peristaltic pump at the rate of 260 $\mu\text{l min}^{-1}$. Approximately twenty 260 μl fractions were collected. SDS-PAGE: Samples were routinely precipitated with trichloroacetic acid (TCA)⁴⁸ at an initial deoxycholate concentration of 0.015% (ref. 49) after their volumes had been adjusted to 1.25 ml with water. Precipitates were washed with 1 ml acetone (at -20°C) and after collection by centrifugation were dried for 15 min at 37 °C. SDS-PAGE was according to Laemmli⁵⁰.

in the presence of detergent (which disrupts the membrane, compare lanes B and C in Fig. 3b). These four proteins must therefore have large domains on the external surface of the vesicle membrane, and indeed they are likely to be entirely peripheral in their association with the membrane as they exist in a soluble form in cytosol²⁵.

Because these four proteins have all the properties of coat proteins, we have designated them as such, but verification must await self-assembly into the non-clathrin coat structure, as in the case of clathrin^{26,27}.

COPs and clathrin coat proteins compared

We noted earlier²⁰ that several Golgi-derived coated vesicle specific proteins were similar in size to the major proteins in clathrin-coated vesicles. It can be seen in Fig. 4 that the electrophoretic patterns of the coat proteins from the two types of vesicles are strikingly similar: the clathrin coat and the COP coat (lanes 1 and 2, respectively) each have a high molecular weight species (clathrin heavy chain, and α -COP), proteins grouped near 100K (adaptins, and β - and γ -COP), and a lower molecular weight species (AP50 and δ -COP). However, the stoichiometries of the various proteins comprising the two coats are different. Clathrin heavy chain is more abundant than the other coat proteins in clathrin-coated vesicles than is α -COP in COP-coated vesicles. This abundance of clathrin is probably an artefact resulting from loss of adaptors (see Discussion) during purification²⁸. In clathrin coats assembled *in vitro*, the relative amounts of clathrin heavy chain and other coat proteins are comparable^{29,30}, with a molar ratio of clathrin heavy chain to adaptin of 3:1 (ref. 31). An approximate determination of COP stoichiometry in the non-clathrin coat by densitometry reveals a ratio of [1]: 2.3:0.9:1.1 (α : β : γ : δ ; α defined as 1). Thus, in

the non-clathrin coat, the 100K proteins (β - and γ -COP together) predominate.

β -COP is an adaptin homologue

We hope eventually to sequence the COPs in order to clone the genes encoding these polypeptides and to raise anti-peptide antibodies. Starting with β -COP, we found its N-terminus to be blocked, and so used internal peptides for sequencing. Rabbit liver Golgi-derived coated vesicle preparations were combined (from ~3 l of cell-free incubations) and subjected to preparative SDS-PAGE; the β -COP bands were extracted and the protein digested with trypsin. A tryptic fragment of β -COP, β -COP 35.39, was isolated by high-pressure liquid chromatography (Fig. 5a), and its sequence determined. It was the same as the sequence in the open reading frame of a previously identified 110K Golgi-associated protein homologous to the β -adaptin protein of clathrin-coated vesicles (R.D. *et al.*, manuscript submitted). The identity is perfect (Fig. 5b), and in the full-length sequence an arginine is predicted just before the sequence of the isolated peptide (underlined in Fig. 5b), as is expected in the generation of peptide fragments by trypsin digestion. This nonapeptide sequence is unique, as it is not found in any of the protein sequences in the National Biomedical Research Foundation (NBRF) database or in the translated nucleic acid sequences

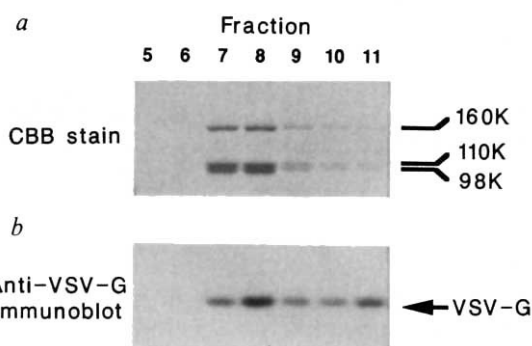


FIG. 2 Localization of VSV-G protein in CHO Golgi-derived vesicles. Gradient fractions from a vesicle preparation using CHO Golgi were subjected to SDS-PAGE; fractions 5–11 are shown. *a*, CBB-stained top portion of the gel. The three major vesicle specific proteins visible in this portion of the gel (indicated on the right) peak once again in fractions 7–9 (42.4–38.6% (w/w) sucrose). The lower relative abundance of the 160 and 110K proteins, here compared to that seen in Fig. 1a, results from electrophoresing the proteins into a 15% (rather than 7.5%) acrylamide gel. *b*, Autoradiograph (region) of lower portion of the gel immunoblotted to detect VSV-G protein (indicated by the arrow on the right).

METHODS. The gradient fractions were obtained and subjected to SDS-PAGE as detailed for Fig. 1. Electrophoretic transfer of proteins from the bottom portion of the resulting gel to nitrocellulose (at 80 V for 4 h) and immunodetection of bound proteins were according to ref. 51. Transferred proteins were visualized by briefly staining with 0.2% Ponceau S/3% TCA; remaining protein binding sites were blocked by incubation in 1% gelatin/1 × PBS/0.1% Tween-20 (blocking buffer; 1 × PBS is 140 mM NaCl/3 mM KCl/3 mM KH_2PO_4 /140 mM Na_2HPO_4). Incubations with antibody were performed at ambient temperature for 1–2 h. The primary antibody, a mouse monoclonal antibody against VSV-G protein⁹, was used diluted 1:1,000 in blocking buffer, and was followed by affinity-purified rabbit anti-mouse IgG (H + L) antibodies (Organon Teknica-Cappel) used diluted 1:3,000 in blocking buffer. After incubation with 0.2 $\mu\text{Ci ml}^{-1}$ ^{125}I -labelled protein A (>30 $\mu\text{Ci } \mu\text{g}^{-1}$; ICN Radiochemicals), the immunoreactive bands were visualized by exposure to Kodak X-Omat AR film using an intensifying screen.

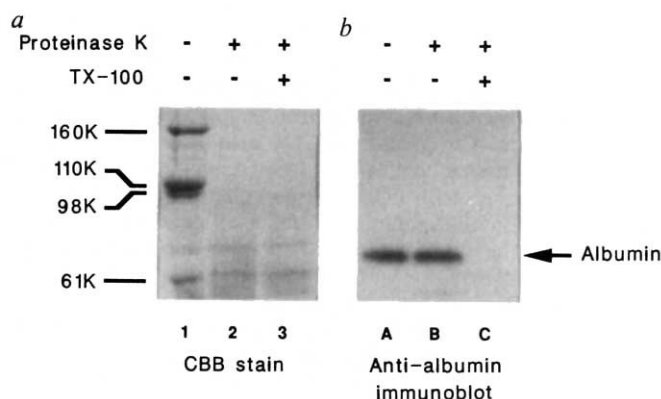


FIG. 3 External orientation of the four major vesicle-specific proteins. Rabbit liver Golgi-derived coated vesicles were incubated with proteinase K and Triton X-100 as indicated, and separate portions of each incubation were subjected to SDS-PAGE. *a*, CBB stain of incubated vesicles, with the four major vesicle-specific proteins indicated on the left. *b*, Autoradiograph of an immunoblot of incubated vesicles to detect albumin, with the albumin band indicated on the right. No albumin band was detected in lane C even after extreme overexposure.

METHODS. The three peak vesicle fractions (7–9) from the final gradient of a rabbit liver Golgi vesicle preparation were pooled, and three 200 μl aliquots were removed. These three samples were each diluted with 1 ml of a 50 mM Tris-HCl (pH 8.0)/5 mM EDTA/3 mM CaCl_2 solution. Proteinase K (Boehringer Mannheim) was added to two samples to 100 $\mu\text{g ml}^{-1}$ from a 1 mg ml^{-1} from a 1 mg ml^{-1} solution in this buffer, and Triton X-100 was added to one of these to 1% (w/v) from a 20% solution in H_2O . Samples not receiving protease or detergent were balanced with buffer and H_2O . Incubations were for 20 min at 37 °C. Following incubation, PMSF was added to all samples to approximately 3 mM from a 100 mM solution in DMSO. After 5 min on ice, deoxycholate was added to 0.015% from a 0.18% solution, and proteins were precipitated by the addition of TCA to 6% from a 24% solution. Triton X-100 was then balanced in samples not yet containing the detergent. The precipitation and subsequent centrifugation yielded a detergent phase, which was diluted to 2 ml with 6% TCA and collected again before a final extraction with 1 ml acetone (–20 °C) to remove the detergent from the precipitated protein. Samples were split (5/8 of each for CBB staining, 2/8 for immunoblotting), subjected to SDS-PAGE as previously described in a minigel (BioRad) apparatus, and immunoblotted to detect rabbit albumin essentially as described in Fig. 2, except that the transfer to nitrocellulose was at 100 V for 1 h, the primary antibody was an IgG fraction of goat anti-rabbit albumin antiserum used diluted 1:3,000, and the secondary antibody was an affinity-purified rabbit anti-goat IgG (H + L) antibody preparation used diluted 1:3,000 (both from Organon Teknica-Cappel).

of the GenBank and European Molecular Biology Laboratory (EMBL) databases. Coupled with the similar molecular weights of β -COP from SDS-PAGE and the cloned protein, this indicates that the two proteins are probably the same.

The nonapeptide could have been derived from the 100K protein present as a minor, non-vesicle-associated contaminant of the coated vesicle fraction, migrating with β -COP during SDS-PAGE. But we used the monoclonal antibody employed in cloning the Golgi-associated protein (M3A5; ref. 21) to show that the 100K protein is as enriched in the non-clathrin-coated vesicles as is β -COP (Fig. 6). A portion of each of the isopycnic gradient fractions from a coated vesicle preparation from rabbit liver Golgi was subjected to SDS-PAGE and either stained for protein (Fig. 6a) or transferred to nitrocellulose and immunoblotted with the M3A5 antibody (Fig. 6b). The M3A5-reactive protein is found in two peaks on the gradient: one corresponds to the peak of the COPs in the coated vesicles (fractions 7–9), and the other (fractions 13–15) is at a much lower density and possibly represents partially uncoated vesicles or other Golgi membrane fragments. The M3A5-reactive 110K protein is, therefore, specific to Golgi-derived coated vesicles. In addition, the immunoblotting reactivity of equal amounts of purified β -COP and the 100K protein is the same (R.D. and T.E.K., personal communication). We conclude that β -COP is the M3A5-reactive 110K protein.

Electron microscopic localization of β -COP

Using an antipeptide antiserum that recognizes the 110K Golgi-associated protein β -COP (anti-EAGE; R.D. *et al.*, manuscript submitted), we used immunoelectron microscopy of incubated Golgi membranes to localize β -COP to the non-clathrin coat structure. As mentioned earlier, when Golgi membranes are incubated *in vitro* with GTP- γ -S, non-clathrin-coated vesicles accumulate. On the other hand, when Golgi membranes are incubated in the absence of active *N*-ethylmaleimide-sensitive fusion protein (NSF)^{32–34}, uncoated transport vesicles accumulate³⁵. Uncoated vesicles are derived from coated vesicles through loss of the coat¹⁸, and fail to fuse in the absence of active NSF. Immunostaining with anti-EAGE confirms the

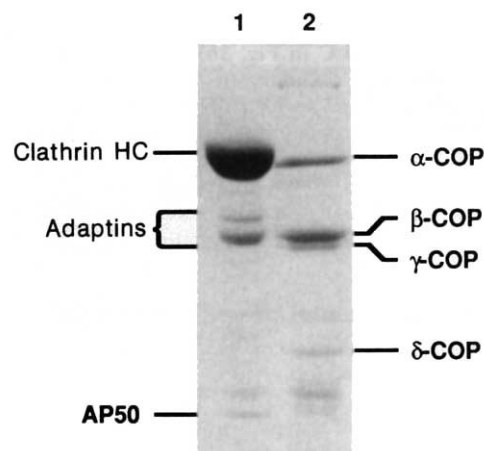


FIG. 4 Comparison of the protein profiles of clathrin-coated vesicles and COP-coated vesicles. Lane 1: Clathrin-coated vesicles, with clathrin heavy chain ('clathrin HC'), the adaptins, and AP50 indicated on the left (AP47 (see Discussion) is too faint to see under these conditions). Lane 2: COP-coated vesicles, with α -, β -, γ - and δ -COP indicated on the right.

METHODS. A clathrin-coated vesicle coat protein extract (10 μ l; lane 1; gift from E. Ungewickell) and one fifth of the peak gradient fraction (fraction 8) of a rabbit liver Golgi-derived vesicle preparation (lane 2) were subjected to SDS-PAGE in a 7.5% acrylamide gel and stained with CBB.

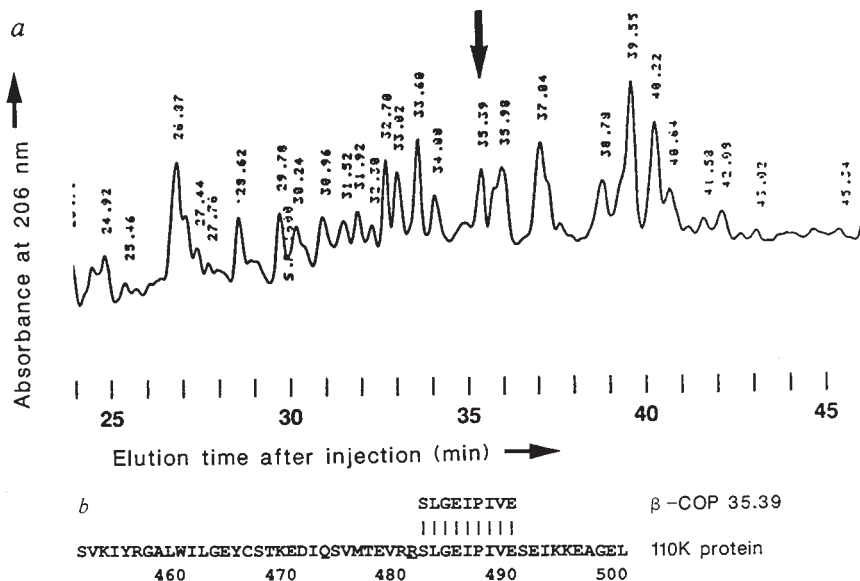
expectation from our biochemical data and the electron microscopy of R.D. *et al.* that β -COP is localized to coated vesicles (Fig. 7a, d, e) and coated buds (data not shown). More importantly, β -COP is absent from uncoated vesicles, which have lost the morphologically defined coat (Fig. 7b, c). This result establishes independently of the biochemical data that β -COP is part of the non-clathrin coat, removed physiologically during the course of uncoating before membrane fusion.

Discussion

The coated vesicle purification procedure reported here is an important step towards the identification of the proteins respon-

FIG. 5 Isolation of a β -COP peptide whose sequence is found within the predicted amino-acid sequence of a previously identified 110K Golgi-associated protein. a, Portion of the chromatograph of the HPLC run of a tryptic digest of β -COP. The peptide eluting at 35.39 min after injection (β -COP 35.39), is indicated with an arrow. b, Sequence of β -COP 35.39, showing perfect match with residues 482–490 in the sequence of the 110K Golgi-associated protein (R.D. *et al.*, manuscript submitted). The arginine residue (residue 481) predicted immediately before the peptide sequence is underlined.

METHODS. Purified vesicles (from 120) 30-ml rabbit liver Golgi preparations were precipitated in TCA and combined for preparative SDS-PAGE in two 1.5-mm-thick, 6% acrylamide gels. Gels were stained with CBB. The bands of β -COP protein were identified by its relative molecular mass (110K), excised, and macerated by forcing them through a fine steel mesh. The protein was extracted by incubating the gel pieces in elution buffer (0.1 M NH_4HCO_3 (pH 8.5)/0.1% SDS) four times for 12 h each. The eluted protein was precipitated (10 min on ice) from the combined eluates with 10% TCA after adding deoxycholate to 0.05%, and the precipitate was collected on a Whatman GF-C glass-filter. After washing sequentially with NH_4OH (25%)/acetone (1:4), ethanol, and ether at room temperature, the precipitated protein was digested for 14 h at room temperature in 0.1 ml digestion buffer (0.15 mg ml⁻¹ trypsin (type IX, Sigma)/0.1 M Tris-HCl (pH 8.0)/2 mM CaCl_2) applied directly to the filter, which had been placed into a microcentrifuge tube. After digestion, the supernatant was recovered by centrifugation through a hole pierced into the bottom of the



tube. This supernatant was filtered through a Millex-GV₄ filter and used directly for HPLC (Merck-Hitachi system with L6200 pump, active mixing chamber, L1000 UV detector, and D2500 integrator) over a Merck Lichrosphere 100 RP 18 reverse-phase column (125/4.0 mm, with endcapped C₁₈ silica, 5- μ m particle, 100-Å pore size) using a gradient of from 10% to 60% acetonitrile in 0.1% TFA over 90 min. Sequencing was performed on an Applied Biosystems 477A gas-phase sequencer with an online PTH-amino acid analyser.

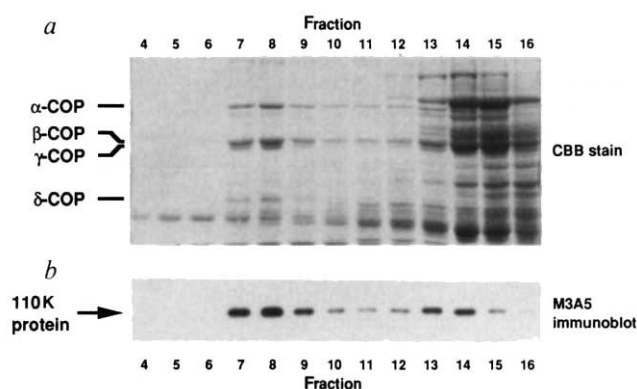


FIG. 6 Localization of the 110K protein to COP-coated vesicles. Gradient fractions were obtained from a rabbit liver Golgi-derived coated vesicle preparation and portions of fractions 4–16 subjected to SDS-PAGE. *a*, CBB stain of a gel in which one fifth of each indicated fraction was electrophoresed. The COPs are indicated on the left. *b*, Autoradiograph (region) of an immunoblot using the monoclonal antibody M3A5. A small fraction (1/250) of each fraction was electrophoresed; the 110K Golgi protein band is indicated with an arrow.

METHODS. SDS-PAGE and immunoblotting were performed essentially as in Fig. 3, except that the primary antibody was M3A5 ascites fluid²¹ used diluted 1:500, and the secondary reagent was that used in Fig. 2.

sible for the events of vesicle budding and targeting. We have used it to identify four subunits of the coat, and to show that one of these, β -COP, is the same as a previously identified protein whose predicted amino-acid sequence is homologous to β -adaptin, a clathrin-coated vesicle coat protein. This homology to β -adaptin not only supports our view that the four COPs are coat proteins, but also shows that the similarity in molecular weights of the constituents of clathrin-coated and COP-coated vesicles is important. The coat of clathrin-coated vesicles consists of clathrin heavy chain (180K) and two related light chains (30–40K), linked to the membrane by adaptor complexes specific for either the *trans*-Golgi network (HA1/AP1) or plasma membrane (HA2/AP2) (refs 12, 13). Each type of adaptor complex is composed of four different polypeptides: β' -adaptin (115K), γ -adaptin (104K), AP47 (47K), and AP19 (19K) for the *trans*-Golgi network-specific adaptor; and β -adaptin (110K or 100K), α -adaptin (110K), AP50 (50K), and AP16 (16K) for the plasma membrane-specific adaptor. The three classes of adaptins (α , β and γ) are all related to each other with about the same degree of homology^{13,36} as β -COP is to β -adaptin (R.D. *et al.*, manuscript submitted), thus defining an extended family of related adaptor proteins involved in the construction of both types of coated vesicles. Within the clathrin coat, the β -adaptins are believed to contact clathrin^{37,38}; given this fact and the similar molecular weights of α -COP (160K) and clathrin heavy chain, α -COP is probably functionally and structurally related to clathrin. Likewise, γ -COP (98K) may be another member of the adaptin family, and δ -COP may be functionally similar to the 47–50K class of adaptor subunits. Also, smaller proteins in the Golgi-derived coated vesicles have molecular weights which indicate that they may be clathrin light chain and AP16/19 equivalents²⁵. If so, a common budding mechanism may underlie the formation of different types of vesicles in eukaryotic cells.

However structurally similar the COPs and the clathrin coat proteins may prove to be, the two types of coats must differ fundamentally in their selectivity. Clathrin adaptors are thought to afford selectivity to clathrin-mediated transport by enabling budding clathrin-coated vesicles to concentrate transported receptors through interactions with the cytoplasmic tails of the receptors^{39,40}. The COP-coated vesicles, being bulk flow carriers, do not selectively concentrate transported cargo. A special system of membrane-associated proteins would therefore seem to be needed to act during vesicle budding to enable the COP coat

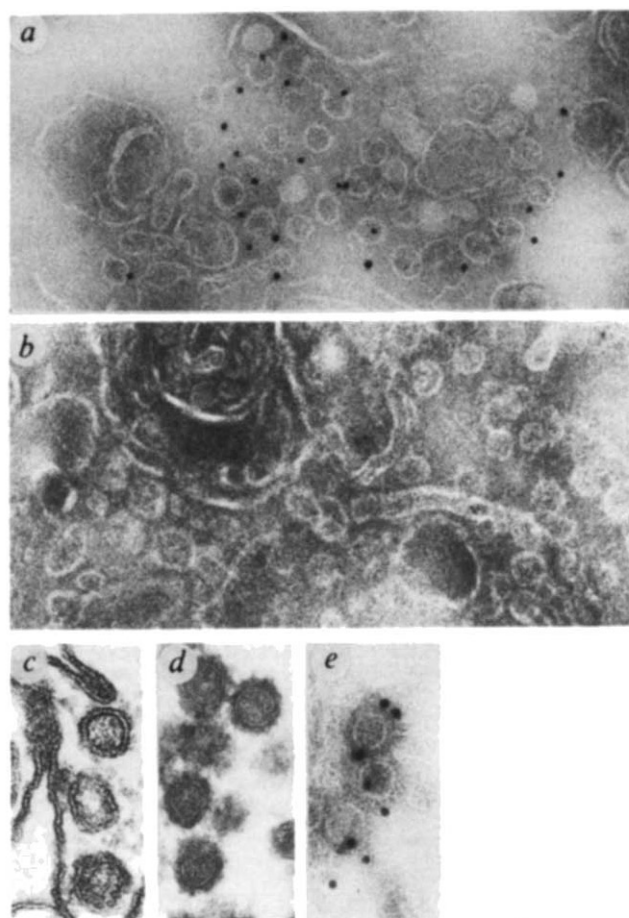


FIG. 7 Immunoelectron microscopic localization of β -COP to the coat of non-clathrin-coated vesicles. CHO Golgi membranes were incubated either with GTP- γ -S to accumulate coated vesicles (*a*, *d*, *e*) or after treatment with *N*-ethylmaleimide (NEM) to accumulate uncoated vesicles (*b*, *c*). *a*, *b*, Immunolabelling using affinity-purified anti-EAGE and protein A/gold performed on ultrathin cryosections. Numerous immunogold particles are associated with the periphery of the non-clathrin-coated vesicles present after GTP- γ -S incubation (*a*), but immunolabelling is absent over the uncoated vesicles accumulated in the absence of *N*-ethylmaleimide-sensitive fusion protein (NSF) activity (*b*). *c*, *d*, Detail of uncoated (*c*) and coated (*d*) vesicles prepared by Epon embedding and tannic acid staining of the incubated membranes used in *b* and *a*, respectively, showing the non-clathrin coat structure present on the vesicles accumulated in the presence of GTP- γ -S (*d*) but lacking on the vesicles accumulated in the absence of NSF activity (*c*). *e*, A detail of coated vesicles (from a sample prepared as in *a*) showing the association of immunogold particles with the coat structure visible here in the cryosection. Magnifications: *a* and *b*, $\times 79,000$; *c*, $\times 118,000$; *d*, $\times 90,000$; *e*, $\times 115,000$.

METHODS. CHO Golgi membranes were incubated with GTP- γ -S as described in Fig. 1 in a final reaction volume of 2 ml for 30 min; for incubation after NEM-treatment, the GTP- γ -S was omitted. Treatment of membranes with NEM was performed as described previously³². After incubation, the membranes were pelleted by centrifugation for 30 min in a horizontal microfuge, and the supernatants were aspirated. The membranes were fixed for 30 min on ice by overlaying the pellets with 1% glutaraldehyde (Polysciences) in 0.1 M phosphate buffer pH 7.4. Ultrathin cryosections were prepared from part of the pellets, and immunolabelling was performed as previously described⁹, except that the primary antibody was affinity-purified rabbit anti-EAGE (R.D. *et al.*, manuscript submitted) diluted 1:100, and it was followed directly by application of the protein A/gold. Epon embedding and tannic acid staining to visualize coats were performed on the remaining portion of the pellets as previously described⁹.

to attach to the membrane, providing a structural equivalent to the multiplicity of receptors packaged into clathrin-coated vesicles. In this regard, we note that a set of proteins ranging from 20–29K is present in quantities substoichiometric with those of α -, β -, γ - and δ -COP, and that, unlike the COPs, these behave as integral membrane proteins during alkaline (carbonate) extraction (data not shown).

Our work also provides a simple rationale for the mechanism of action of brefeldin A. Brefeldin A is a fatty-acid derivative of fungal origin⁴¹ which blocks the forward transport of secretory proteins from the endoplasmic reticulum to the Golgi apparatus^{23,24} and causes a disassembly of the Golgi apparatus⁴² with the return of its contents to the endoplasmic reticulum^{43,44}

in a microtubule-dependent process⁴⁵. The earliest action of brefeldin A (within 30 s) is to cause release of the 110K protein (β -COP) from the Golgi apparatus, an event which precedes any morphological changes²². The effects of brefeldin A on transport can therefore be rationalized as a cessation of the forward flow of membrane which is normally mediated by COP-coated vesicles, caused by preventing assembly of the coat onto the membrane. Retrograde transport back to the endoplasmic reticulum from the Golgi^{43,44,46,47}, not relying upon COP-coated vesicles, would continue, resulting in the transport of the membranes of the Golgi back to the endoplasmic reticulum. The Golgi apparatus may well exist on the razor's edge of a steady-state equilibrium. □

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LETTERS TO NATURE

Ejection of pulsars and binaries to the outskirts of globular clusters

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THREE-BODY interactions can eject stars, singly or in binaries, from the core of a globular cluster to its outskirts, whither dynamical friction may take more than 10^8 yr to return them. We show here that such a process can explain why the binary pulsar 2127+11C in the cluster M15 (and perhaps 1744–24A in Terzan 5) is now far from the cluster core. A suitable encounter could have given the pulsar enough velocity to eject it to its present position, and also replaced its original stellar companion with a neutron star. For ejection of PSR2127+11C to be likely, the core of M15 must be composed of heavy degenerate stars at a density greater than 10^7 pc⁻³, maintained for $\geq 10^8$ yr; this is consistent with previous dynamical estimates. A natural combination of factors enables us to see 2127+11C: a binary of longer period could not have received a large enough impulse to escape the core, whereas one of shorter period would have been ejected from the cluster or would have collapsed because of orbital decay by gravitational

radiation. Pulsars and pulsar binaries ejected from clusters will contribute to the birth rate of recycled pulsars in the inner Galaxy.

In thermal equilibrium, neutron stars and binaries, being heavier than the unevolved majority of stars in M15, would be found at the bottom of M15's potential well. Indeed, the low-mass X-ray binary AC211 (refs 1, 2) and the four single radio pulsars 2127+11A,B,D,E (ref. 3) are all within 0.4 pc of the optical centre of M15 (the ultraviolet U-band core radius of which is $r_0 < 0.1$ pc (ref. 4), where the core radius is the radius at which the surface density of a specified population of stars is half the central value). To reach its current position⁵, projected 2.7 pc from the core of M15, the binary containing PSR2127+11C must have been ejected with a speed ≥ 50 km s⁻¹ from the high-density central region where it formed (Fig. 1).

PSR2127+11C has a spin-down age $\tau_c = P/2\dot{P} = 1 \times 10^8$ yr (ref. 5). This suggests that the pulsar was spun up by accretion not much more than τ_c ago. Assuming, as suggested by the mass function, that the binary consists of two $1.4 M_\odot$ neutron stars, gravitational radiation will cause the two neutron stars to merge 2×10^8 yr from now. Integrating the orbit backwards from the present orbital period and eccentricity ($P_b = 8.05$ h, $e = 0.68$) under the influence of gravitational radiation alone, 10^8 yr ago the binary would have had $P_b = 12$ h and $e = 0.75$, and 2×10^8 yr ago $P_b = 16$ h and $e = 0.79$. A binary consisting of two compact objects offers no opportunity for accretion or tidal capture. Therefore either the current companion must have been exchanged for the non-degenerate star from which the pulsar

was accreting, or the pulsar must have been spun up elsewhere, and exchanged into a binary already containing the current companion. We begin with the first possibility.

Because M/L , where L is luminosity, rises steeply⁶⁻⁸ for $r < 0.1$ pc in M15, most of the stars encountered there by a binary will be heavy degenerate stars. If star 3 encounters a binary of semimajor axis a_i (consisting of stars 1 and 2) and exchanges with star 2, the recoil velocity v_{13} of the final binary consisting of stars 1 and 3 with semimajor axis a_f is

$$\left(\frac{v_{13}}{v_c}\right)^2 = \frac{M_2 M_3 (M_1 + M_2)}{(M_1 + M_3)(M_1 + M_2 + M_3)^2} \times \left[\frac{a_i}{a_f} \frac{M_3}{M_2} - 1 + \left(\frac{v_{in}}{v_c}\right)^2 \right] \quad (1)$$

where v_c , given by

$$v_c^2 = \frac{GM_1 M_2 (M_1 + M_2 + M_3)}{a_i M_3 (M_1 + M_2)} \quad (2)$$

is the relative incoming velocity $v_{in} = (v_3 - v_{12})$ for which the system would have zero total energy (G is the gravitational constant). The $(v_{in}/v_c)^2$ in equation (1) may be neglected for hard-X-ray binaries, so a_i and the period P_i of the initial binary are uniquely determined by the recoil velocity and the period P_i after exchange. If the pulsar was accreting from its erstwhile companion, then $M_2 < 0.7 M_\odot$, the mass of stars just leaving the main sequence. The companion cannot have been very light (such as the $0.02 M_\odot$ companion of PSR1957+20), because if $M_2 \ll M_1 = M_3$, most of the exchange cross-section produces only wide ($a_f \approx (M_3/M_2)a_i$), extremely eccentric ($1 - e_f \approx M_2/M_3$) binaries with negligible recoil, $v_{13} \approx 0.3(M_2/M_3)^{1/2}(GM_3/a_i)^{1/2}$. We therefore consider an intermediate mass $M_2 = 0.56 M_\odot = 0.4 M_1$. For $P_i = 12$ h (encounter τ_c ago), a recoil exceeding 50 km s^{-1} requires $P_i > 8$ h; $P_i = 8$ h (encounter very recently) requires $P_i > 4$ h. In the latter case, a normal main-sequence star could have filled its Roche lobe. The pre-encounter orbit will have been circularized by tidal dissipation. As part of a larger investigation of the interaction of cluster binaries with compact objects, we have calculated cross-sections for subsequent exchanges. Our simulations show that the median post-encounter orbit has eccentricity $e_f = 0.7$ (and approximately

$d\sigma/de_f \propto e_f$), like that of PSR2127+11C. The cross-section for exchange with recoil velocity in range dv_{rec} for such a binary (with $P_i = 8 P_8$ h) gives a rate of reaction with heavy degenerate stars of $v_{in} = 10 v_{10} \text{ km s}^{-1}$ and density $n_d = 10^6 n_{d6} \text{ pc}^{-3}$ of

$$n_d \left(\frac{d\sigma}{dv_{rec}} \right) v_{in} = 0.8 \left(\frac{d\sigma}{dv_{rec}} \right) P_8^{2/3} v_{10}^{-1} n_{d6} \text{ per } 10^{10} \text{ yr} \quad (3)$$

where $d\sigma/dv_{rec}$ is shown in Fig. 2. Treating the bodies as point masses, the total cross-section for exchange is $\bar{\sigma} = 7$. For $P_i = 8(12)$ h, recoil velocities exceeding 40, 50 and 65 km s^{-1} have $P_8^{2/3} \bar{\sigma} (> v_{rec}) = 2.4$ (2.6), 1.4 (1.6) and 0.7 (0.5), respectively. Recoil velocities exceeding 70 km s^{-1} have negligible cross-section. Some 55% of the exchanges are affected by collisions between the main-sequence star and one of the neutron stars (see Fig. 2); all encounters leading to tidal interactions with the main-sequence star have cross-section $\bar{\sigma} \approx 10$ (naïve extrapolation of results from encounters of bodies of equal mass^{9,10} would overestimate recoil velocities and the importance of tidal interactions, because exchange of unequal masses results in significant expansion of the orbit). We note that recycled pulsars in exchange binaries will not have spins $\mathbf{S}$ aligned with the binary orbital angular momentum $\mathbf{L}$ (as expected in binaries where mass transferred from the companion spun up the pulsar). They are thus good candidates for observing geodetic precession of the pulsar spin. But exchanges do not destroy all memory of the initial binary. If $\mathbf{S} \parallel \mathbf{L}$ in the initial binary, some 65% of exchange binaries have $\mathbf{S} \cdot \mathbf{L} > 0$, because prograde encounters are more efficient than retrograde ones in causing exchanges.

The radial distribution of pulsars in M15 suggests that the half-mass radius of neutron stars (and other dark remnants of similar mass) is $r_{dh} = 0.2$ pc. The negative values of $\dot{P}$ of pulsars 2127+A,D require⁸ that the density of dark remnants rises more steeply than $n_d \propto r^{-2}$ from 0.2 pc to 0.1 pc, where $n_d(0.1 \text{ pc}) = 10^6 \text{ pc}^{-3}$. That the density continues to rise to at least 10^7 pc^{-3} is suggested both by the apparently high velocity dispersion^{6,7} at < 0.05 pc and by models of the post-core collapse evolution of clusters. These predict¹¹ that even during the maximum expansion of core oscillations, the central density of heavy remnants $n_d(0) > 10^7 \text{ pc}^{-3}$. Binaries, being heavier, will sink to the core between encounters that eject them. Equation (3) shows that the time between encounters that eject the binaries at least

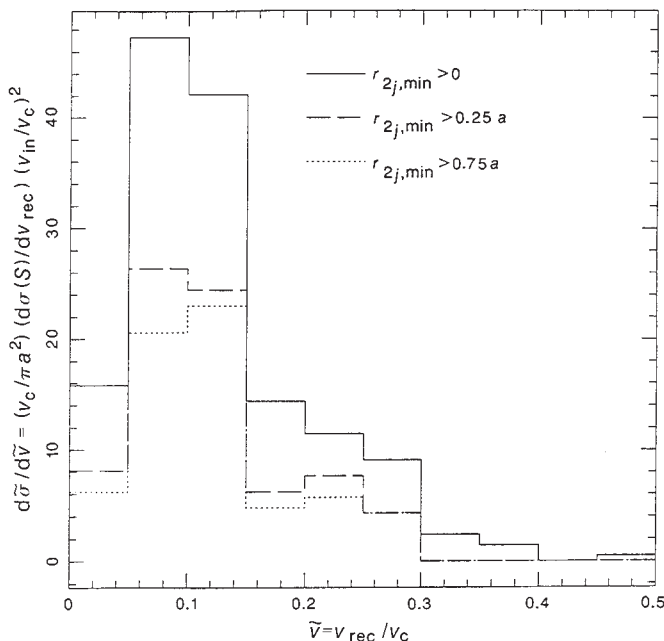


FIG. 1 *a*, Turning points of the orbit of a body ejected at speed v_{rec} from radius r_i in the core of a single-mass $W_0 = 12$ ($c = 2.74$) King¹⁶ model cluster of core radius r_0 and line-of-sight central velocity dispersion σ . Form of the curve is independent of r_0 and W_0 for $r/r_0 \ll 100$. For PSR2127+11C to reach its projected distance of 2.7 pc from the core of M15, it must have been ejected at $\geq 50 \text{ km s}^{-1}$, a result confirmed by more sophisticated cluster models⁸. *b*, Timescale for dynamical friction to carry back to the core a star or binary system of mass M_b ejected with speed v_{rec} from radius r_i . The form of the curve is independent of r_0 for $r_i \geq r_0$. Friction time on the ordinate is labelled for $M_b + \langle m \rangle$ equal to 10^{-5} of the cluster mass M_{cl} ($\langle m \rangle$ is the mean mass of cluster stars) but scales inversely with $M_b + \langle m \rangle$. Friction time is determined largely by the density at pericentre. Perpendicular velocity kicks from cluster stars passing at the outer reaches of the orbit can shift the pericentre of the nearly radial orbit of the ejected body, creating a distribution of sinking times with a tail extending to times much larger than indicated by the curve, which neglects such kicks. Width of this distribution of sinking times becomes significant for orbits with initial apocentric distances $r_a > 4(M_b + \langle m \rangle)/\langle m \rangle$ times the initial pericentric distance, where $\langle m \rangle$ is the mean mass of cluster stars encountered near r_a . Timescale for the binary pulsar 2127+11C to sink in M15 is $\sim 5 \times 10^7 (r_p/0.05 \text{ pc}) \text{ yr}$. Timescale for the binary pulsar 1744-24A to sink in Terzan 5 is $\geq 1 \times 10^8 \text{ yr}$. Uncertainties in the cluster structure, the radii from which the binaries were ejected, and the true r_a make the estimates of sinking time uncertain to at least a factor of five. Neither system's pericentric distance is likely to have been shifted substantially. Ejected pulsars are most likely to be found near the half-mass radius (3.5 pc for M15): convolution of Figs 1a and 2 shows that few pulsars will be found beyond the half-mass radius ($85 r_0$), because only a narrow range of ejection velocities have apocentres there. Few will be found inside the half-mass radius, because there friction will rapidly drag them back to the core⁸.

as far as PSR2127+11C is $\leq 10^9$ yr. The mean number of such ejected binaries observed at any one time is

$$\langle N_{ej} \rangle \approx N_{prog} \langle n_d \rangle \sigma v_{in} T_{ej} \quad (4)$$

where N_{prog} is the mean number of progenitor binaries in the core at any time, T_{ej} is the lifetime of the ejected binary, and $\langle n_d \rangle$ is the time-average density through which the progenitor binaries move. M15 contains one low-mass X-ray binary ($N_{prog} = 1$) and $T_{ej} \approx 10^8$ yr (see Fig. 1b), so this gives $\langle N_{ej} \rangle \approx 0.1$. Given the uncertainties in the central density, it seems to be possible that PSR2127+11C was ejected in this way by an encounter between a heavy remnant and a low-mass X-ray binary. If so, not many more such systems will be found in M15 or in the other ~ 20 post-core-collapse clusters¹², which contain between them only 10 low-mass X-ray binaries.

The long periods of the pulsars found in M15 suggest that they may have been spun up not by gradual Roche-lobe overflow in a binary, but by accretion of the remains of a tidally disrupted star. If a fraction ϵ_{ak} of the accretion energy were absorbed in the disrupted remains and converted to kinetic energy, accretion of only $10^{-4} M_{\odot} / \epsilon_{ak}$ would unbind the rest of the material, yet would provide enough to spin the neutron star (moment of inertia $10^{45} I_{45} \text{ g cm}^2$) up to equilibrium spin for periods

$$P > 50 (\epsilon_{ak}/0.1)^{3/4} I_{45}^{3/4} \text{ ms} \quad (5)$$

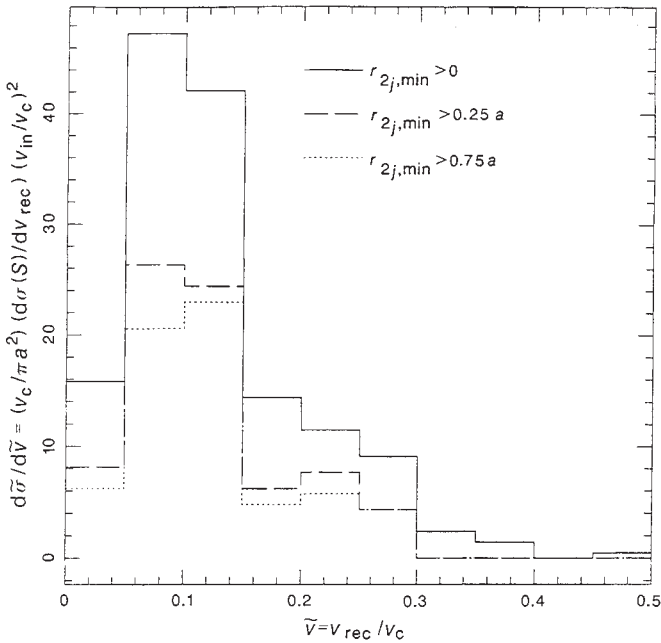


FIG. 2 Differential cross-section of binary recoil velocity for exchanges in which a passing heavy star is exchanged for the lighter star of a binary. Final binary consists of two stars of mass $1.0M_h$; expelled star has mass $0.4M_h$. Here $M_h = 1.4M_{\odot}$, appropriate for neutron stars or heavy white dwarfs. Initial binary is circular, with semimajor axis a ; v_c , defined in the text, is 273 km s^{-1} for $P_1 = 8 \text{ h}$. Light star was treated as a point mass for the solid curve; dashed and dotted curves show only exchanges in which collisions between a light star of finite radius and either of the heavy stars were avoided. $r_{2j,min}$ is the minimum distance between star 2 and either of the other stars during the encounter. For $P_1 = 8 \text{ h}$, the two collision criteria correspond to passage of a main-sequence star of radius $R_* = 0.56 R_{\odot}$ within $1.07R_*$ and $3.2R_*$ (within which tidal dissipation would modify the orbit) of one of the neutron stars. Total cross-section for heavy-star exchanges that leave the light star in the binary is smaller by a factor of six. Cross-sections shown are based on Monte Carlo simulations of 1,000 three-body interactions, all integrated to completion. 302 of the interactions contributed to the exchange channel shown here. In the simulations, v_{in} was selected uniformly in the range $0.05 < v_{in}/v_c < 0.15$; the results depend only weakly on v_{in}/v_c , provided that it is small.

The lifetimes of such systems could be very short, $< 10^6$ yr, permitting a rate of pulsar formation as high as 100 per 10^8 yr while having an expectation value of less than one system being caught in the act (AC211 may be a longer-lived system contributing negligibly to the pulsar birth rate). This would resolve the discrepancy between the pulsar birth rate and that of low-mass X-ray binaries¹³.

We therefore consider the second possibility: that the pulsar was spun up by the remains of a disrupted star, and subsequently exchanged into a binary containing its present companion. Exchanges into binaries containing a heavy degenerate require, for $P_1 = 11 \text{ h}$, $P_2 = 15 \text{ h}$ for $v_{rec} = 50 \text{ km s}^{-1}$ ($\tilde{\sigma}(>v_{rec}) = 0.8$) and $P_1 = 30 \text{ h}$ for $v_{rec} = 80 \text{ km s}^{-1}$ ($\tilde{\sigma}(>v_{rec}) = 0.2$). The exchange rate is given by equation (3), but with the coefficient (which depends on the mass ratios) increased by a factor of 2.4. The initial eccentricity of the orbit affects $\tilde{\sigma}$ only weakly. If not too eccentric, such initial binaries have lifetimes against gravitational radiation approaching the age of the Universe. Binary-binary interactions have similar cross-sections and recoil velocities. The expected number of ejected systems is then $\langle N_{ej} \rangle = N_{PSR} \langle n_{db} \rangle \sigma v_{in} T_{ej}$, where $\langle n_{db} \rangle$ is the time-average density of degenerate binaries through which the pulsars passed. As $N_{PSR} \approx 3$ (we count only single pulsars that are in the dense core and as bright as PSR2127+11C, because only they could give rise to that system), this scenario gives $\langle N_{ej} \rangle \approx \langle n_{db} \rangle / 10^7 \text{ pc}^{-3}$. In their 10^8 -yr lifetime, relaxation will spread single pulsars over $\sim 0.1 \text{ pc}$, where $\langle n_{db} \rangle \approx 10^6 \text{ pc}^{-3}$. Thus this possibility is much less probable than that involving a low-mass X-ray binary, unless the inner 0.1 pc of M15 is composed largely of tightly bound degenerate binaries.

Could PSR1913+16, a pulsar in our Galaxy similar to PSR2127+11C, have been created in a globular cluster, with a recoil slightly larger than, rather than slightly lower than, the escape velocity? The seemingly large proper motion¹⁴ of PSR1913+16 is consistent with this hypothesis. Difficulties with models creating PSR1913+16 in supernovae¹⁵ make a cluster origin attractive. But the birth rates pose a difficulty. PSR1913+16, with a lifetime of $\sim 10^{8.5}$ yr, was discovered in a survey covering 168 deg^2 along $1/20$ of the galactic plane; the birth rate is thus $\sim 10^{-7} \text{ yr}^{-1}$ if objects like it are found only within 300 pc of the galactic plane. It lies 6.5 kpc (based on the dispersion measure) from the Galactic Centre. The vast majority of the clusters with high central density lie within a projected distance of 2.5 kpc of the Galactic Centre¹². A recoil velocity $v_{ej} > \sqrt{v_{ri}^2 + v_{esc}^2}$ would be required to escape (v_{esc} one of these clusters, and then rise ($v_{ri} > 100 \text{ km s}^{-1}$) the required distance in the galactic potential. Such recoil velocities cannot be obtained in physically reasonable three-body encounters, so it is unlikely that PSR1913+16 could have been ejected from one of those clusters. Possible sources are thus the rare halo post-core-collapse clusters (like M15), or clusters of modest central density. Then objects like PSR1913+16 could be found anywhere within $\sim 10 \text{ kpc}$ of the Galactic Centre, suggesting a birth rate of $\sim 10^{-6.4} \text{ yr}^{-1}$, but this seems hard to maintain from the rare dense clusters at large galactocentric radius. A birth rate an order of magnitude lower might be maintained, however, so we are reluctant to reject this idea using rates derived from statistics of single objects. In any case, there are likely to be several systems like PSR1913+16 in the inner 2.5 kpc of the Galaxy, ejected from the dense clusters there. Similar recoil can occur in hard binaries containing white dwarfs (cataclysmic variables), complicating the task of determining membership of candidates far from cluster centres^{11,17}.

Note added in proof: Recent U-band images from the Hubble Space Telescope¹⁸ show that M15 has $r_0 \approx 0.1 \text{ pc}$, and Meylan, Dubath and Mayor (abstract, American Astronomical Society) find the central velocity dispersion to be nearly constant at 14 km s^{-1} , in contrast to ref. 7. The parameters chosen for Fig. 1 thus seem almost perfectly appropriate to M15. $\square$

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Solar-cycle dependence of the Sun's deep internal rotation shown by helioseismology

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HELIOSEISMOLOGY, the study of solar oscillations, yields information on the Sun's internal rotation and magnetism which is of great importance in understanding the 22-year solar cycle. We show here that helioseismic data suggest that the Sun's internal rotation rate, at depths greater than half the solar radius, has changed systematically during the most recent cycle. There is no variation, however, in the rotation over a range of intermediate solar radii covering the upper part of the Sun's radiative interior and the lower part of the convective zone; this intermediate region is where, according to the same helioseismic data, an abrupt change in rotation rate with depth accompanies the transition from convective to radiative structure. We suggest that the modulation of the rotation rate in the Sun's interior could be caused by a torsional oscillation, provided that a poloidal magnetic field of kilogauss strength exists in the radiative interior.

Kuhn¹ has argued that the symmetric part of the fine structure in oscillation data has systematically changed during the current cycle. Libbrecht and Woodard², in particular, have demonstrated that a perturbation acting very near to the solar surface is the primary source of the sizeable differences in the symmetric parts of the fine structure between oscillation data gathered at Big Bear Solar Observatory in the summer of 1986 at solar minimum, and that from the summer of 1988 when the solar surface was considerably more active. Here we use the anti-symmetric part of the fine structure (rotational frequency splittings) in the available oscillation data to learn what we can about the Sun's internal rotation through the activity cycle.

Because we know the rotation rate in the Sun's equatorial plane best, this will be our tracer of any radial and/or temporal changes. We first compare the rates deduced from the two data sets of Libbrecht and Woodard (ref. 2 and personal communication). These two data sets are of special interest because they were gathered with the same instrumentation, reduced using the

same analysis procedure and span the same range of the oscillation spectrum. In Fig. 1, we show two rotation rates, in the plane, calculated using the optimal averaging³ method of inversion. The r.m.s. error bars for the rotation rates from the 1988 data (not shown) are slightly smaller than the corresponding error bars from the 1986 data. The radial error bars represent the radial spatial resolution of the data as given by the half-width at half-maximum of the resolving kernels, which have gaussian-like forms. The radial error bars from each data set are virtually identical, point by point. The two rotation laws are most similar between ~ 0.5 of the solar radius R and the base of the convection zone—roughly the region that these oscillations sample best. Also, above $0.5R$ each point shown is very nearly statistically independent of its neighbours. In the convection zone, the laws are not quite so similar, but they differ by $<1\sigma$ everywhere with some tendency for the 1988 rate to be faster. Both rates near $0.9R$ are consistent with the surface equatorial rate as most recently determined by Stenflo⁴ from magnetic features. The two laws differ most beneath $0.5R$, where our knowledge is less secure. In the deep interior the difference is significant at the 1.5σ level. The two innermost points are not quite statistically independent. The abrupt increase in rotation in the interior depends critically on the low-degree splittings in the 1986 data. This abrupt change is more secure than the modest increase found⁵ using an earlier version⁶ of the 1986 data. This earlier version lacks the lowest-degree splittings present in the current form of the data. Our faith in the reality of the change in rotation in the interior would be strengthened if other data sets yielded a similar pattern.

We previously suggested⁷ the possibility of such a change occurring between 1983 and 1986. We now have data from two other data sets, both taken past the minimum—the second taken by Jefferies *et al.* (ref. 8 and personal communication). Figure 2 shows the rotation rate at $0.4R$ as a function of time. This radius was chosen because all available data sets sample fairly well to this depth. We used a constrained least-squares method to solve the inverse problem for rotation from each set of splitting data. The constraint is against a gradient in rotation. Although this approach tends to minimize the differences in rotation laws near $0.4R$, the difference between the results from the 1986 and 1988 sets persist. We used a least-squares approach because some of the data sets were averaged over the radial order and consequently, the resulting optimally averaged kernels are not sufficiently localized. The inverse number of sunspots is also shown in Fig. 2. This number is calibrated to the two data sets from Big Bear Solar Observatory. The earliest of the several sets used is from 1983.4 and is due to Duvall and Harvey⁹. The point at 1984.6 is a weighted composite of the rotation from the data

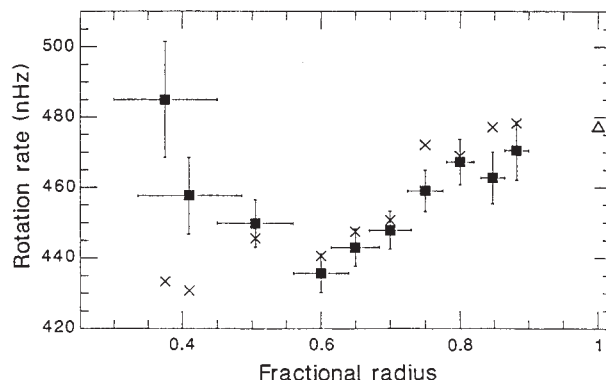


FIG. 1 Two internal rotation laws for the Sun's equatorial plane from the 1986 and 1988 data of Libbrecht and Woodard². ■ and X, rate from the 1986 and 1988 data, respectively; △, surface equatorial rate from magnetic features⁴. The vertical error bars are the r.m.s. errors from the optimally averaged³ calculation. The horizontal error bars span the full width at half-maximum of the gaussian-like resolution kernels revealing the granularity of the rotation laws.

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of Brown and Morrow¹⁰, Tomczyk¹¹ and Rhodes *et al.*¹². The rotation rate in the deep interior seems to show a maximum at solar minimum and seems to be anticorrelated with the sunspot number. This correlation forced us to consider the possibility that a surface perturbation due to the sunspots has been misinterpreted as a change of rotation in the interior. We did not find any such effect because we could not imagine how sunspots could preferentially perturb the low-degree splittings in the antisymmetric part of the data. The three critical points in the trend shown in Fig. 2 depend on low-degree splittings in the data sets that were not averaged over radial order—the sets from 1983.4, 1988.5 and especially 1986.5. Above $0.6R$, the rotation laws from all the sets in Fig. 2 are quite similar. In particular, at $0.7R$, the base of the convection zone, the rotation rates deduced from the aforementioned data sets are nearly identical.

Considering further the region near the base of the convection zone, it is not unreasonable to think that it is the centre of a sharp transition from essentially surface-like differential rotation above to solid-body rotation beneath. After all, the transition in Fig. 1 is very nearly spread over the resolution width of the data at $0.7R$. We have previously shown⁷ that if a single discontinuity in rotation is allowed at any radius, the gradient is by far the largest if the discontinuity is at the base of the convection zone. In particular, using the 1986 data of Libbrecht and Woodard², and fixing the jump at $0.7R$, the rate just above the base of the convection zone is

$$\Omega(\theta, 0.7R+) = (462.4 \pm 5.8) - (60.5 \pm 13.1) \cos^2 \theta - (81.7 \pm 15.6) \cos^4 \theta \quad \text{nHz} \quad (1)$$

where θ is the polar angle for the Sun. This rate is very close to the observed surface differential rotation. On the other hand, the rate just beneath $0.7R$ is

$$\Omega(\theta, 0.7R-) = (438.7 \pm 3.7) - (11.2 \pm 18.3) \cos^2 \theta - (9.8 \pm 26.6) \cos^4 \theta \quad \text{nHz} \quad (2)$$

implying θ -independent rotation. Equations (1) and (2) illustrate that we know the rotation rate in the equatorial plane best. We would obtain nearly the same results for rotation from the summer 1988 data. We emphasize, however, that including the discontinuity does not change the trends in the deep interior's rotation shown in Figs 1 and 2. Thus, there might be no variation in rotation just below the base of the convection zone (~ 0.6 – $0.7R$) even with variations beneath.

From the helioseismic data, we infer that the Sun's rotation in the outer part of the radiative interior may well be spatially and temporally constant. In the deeper interior, however, there is evidence for changes in its radial dependence occurring on

a timescale of years. Unfortunately, at this time we can say nothing about the θ -dependence of these changes. The evidence for the rapid variation in rotation is not statistically compelling. A conservative perspective might be that all the differences in the interior rotation presented here reflect merely the true uncertainty of the measurements. From this perspective, the most important result of our analysis would be the constancy of the rotation at the base of the convection zone. As well, in the region beneath $0.5R$ we could not exclude an inward rise in the rotation rate as suggested by the 1986 data. Such a rise would be important for understanding the angular momentum evolution in stars.

If we consider the rapid variation in rotation to be real, we note that because it occurs far from the convective region its origin could only be in a magnetic torque. A long time ago, Walen¹³ suggested that the excitation of torsional oscillations throughout the Sun may be responsible for solar activity. Torsional oscillations have been discussed recently by Gough¹⁴ in a different context. These oscillations occur in the presence of a poloidal magnetic field, $\mathbf{B}_p$, whereby a toroidal field and differential rotation exchange energy.

The equation for the oscillation of the angular velocity Ω in the radiative interior is

$$\rho r \sin^2 \theta \frac{\partial^2 \Omega}{\partial t^2} = \frac{1}{4\pi} (\mathbf{B}_p \cdot \nabla) [r^2 \sin^2 \theta (\mathbf{B}_p \cdot \nabla) \Omega] \quad (3)$$

where ρ is the local density. We ignore dissipative effects because their timescale is so much greater than a few years. For the time-dependent part of the toroidal field, the ϕ -component of the induction equation gives

$$\frac{\partial B_\phi}{\partial t} = r \sin \theta (\mathbf{B}_p \cdot \nabla) \Omega \quad (4)$$

To force equation (3) to predict the observed time and presumed spatial independence of Ω in the outer radiative zone, we require that $\nabla \Omega$ vanish at the outer edge of the zone and that B_p^2/ρ be relatively large in that region. The torsional oscillation in Ω would be observable deeper down if B_p^2/ρ were smaller there. Thus, unlike a core relic field, the B_p field should be relatively more intense near the base of the convective envelope. Further, if the 22-year periodicity of the cycle were due to the lowest-order mode of the torsional oscillation, then $\langle B_p \rangle \approx 1$ kG. With this and equation (4) and the assumption that the change in rotation rate has an amplitude of 20 nHz, the toroidal field would have an amplitude of ~ 10 kG. Such an amplitude would be too small to cause a visible change in the spectrum of solar p-modes (so called because pressure is the dominant restoring force). Thus, we could see the torsional oscillation only by the change in rotation it would induce.

The essentially constant megagauss toroidal field centred near the base of the convection zone that we have inferred helioseismically (ref. 15 and manuscript in preparation) has no role in the oscillation. This field could, however, be related (by a dynamo action) to the constant B_p required here. Anyway, that toroidal field has too much energy stored in it to vary over the activity cycle.

Thus, the picture of the Sun's activity emerging from these helioseismic data is different from the commonly accepted one. That is, the 22-year cycle is due to the excitation of a torsional oscillation in the radiative interior that introduces a disturbance in the magnetic state of the envelope. There are many unclear points in this suggestion but our picture can be observationally tested in the near future. In particular, we expect that the decrease in the equatorial rotation rate near $0.4R$ will continue through the solar maximum. In addition, to conserve angular momentum, it would be expected that the oscillation in Ω would have the opposite sign deeper down. Improvement in the signal-to-noise ratio of data for the deeply penetrating, low-degree solar oscillations will allow this to be checked. Also, better higher-degree data will be available in the future. With these

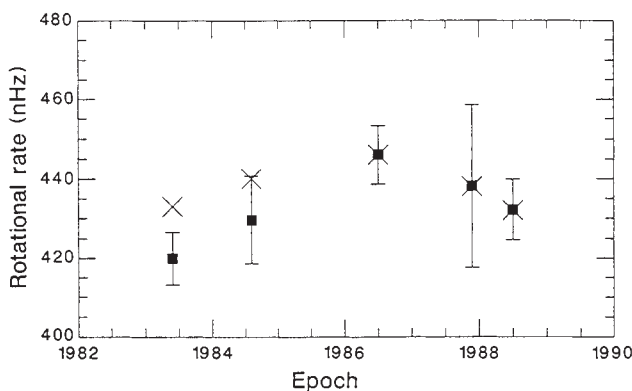


FIG. 2 The rotation rate at $0.4R$ in the equatorial plane as a function of time. ■, rates from least-square calculations; X, inverse of the mean sunspot number for the epoch of each data set, as calibrated to the rates from the 1986 and 1988 data of Libbrecht and Woodard².

data, we will be able to determine whether the rotation rate in the envelope is really constant. The antisymmetric part of all the data may prove to be a more useful probe of the magnetic activity in the envelope than the symmetric part. □

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Fluorescence spectroscopy and spectral diffusion of single impurity molecules in a crystal

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MOTIVATED by the possibility of studying individual local environments in the solid state, there have been attempts to observe the optical absorption spectra of single impurity molecules trapped in crystals^{1,2}. For example, time-dependent shifts in spectral features (spectral diffusion) are expected to result from motions of the molecules surrounding the impurity species. Recent advances in high-efficiency fluorescence excitation spectroscopy using ultra-thin sublimed crystals³ have now removed the earlier obstacle of low signal-to-noise ratios. Here we report the observation of jumps in the resonance frequency, on timescales of seconds to minutes, in the fluorescence excitation spectrum of single molecules of pentacene in crystals of *p*-terphenyl cooled to 1.5 K. These effects are seen only for some impurities, which probably correspond to pentacene molecules in particularly strained local environments; most impurities show no time-dependent behaviour. We speculate on the possible causes of these spectral jumps, although further work will be required to draw definitive conclusions about the molecular motions involved.

In our fluorescence excitation technique, a tunable dye laser (1 MHz linewidth) was used to excite the 0–0 electronic transition of pentacene substitutional impurities in the *p*-terphenyl crystal. As the excitation wavelength was varied, the emitted fluorescence that passed through a long-pass filter was measured with a photon counter. Such methods are capable of measuring the extremely small absorption from single ions in traps⁴, but in a single-molecule experiment in a solid the scattered light from the sample and other components must be reduced to very low levels. This was achieved by limiting the excited volume through using 1–10- μ m-thick sublimed single-crystal samples and laser focal diameters of 5 μ m full width at half maximum (FWHM). Following Orrit *et al.*³, the Stokes-shifted fluorescence was collected with high efficiency using optics with a numerical aperture of 0.98.

The crystals were cooled to 1.5 K, at which temperature the homogeneous linewidth, $\gamma \approx 7.5$ MHz (ref. 5), of individual pen-

tacene defects in the O₁ site of the crystal (there are four principal sites for pentacene substitution, one for each of the four *p*-terphenyl molecules in the unit cell⁶) is much less than the inhomogeneous line width ($\Gamma_1 \approx 2$ –42 GHz, depending on strain in the crystal). This well-known inhomogeneous broadening effect⁷ occurs for all zero-phonon transitions of defects in solids at low temperatures⁸. The inhomogeneous line profile represents the centre frequency distribution of the pentacene guests produced by random strain and electric fields present in the crystal. For single-molecule spectra, the laser may be tuned far into the wings of the inhomogeneous line, where the number of defects per homogeneous width is less than one—that is, where non-overlapping individual pentacene defects may be found. For most measurements, the pentacene concentration was approximately 6.6×10^{-8} mol mol⁻¹, and $\Gamma_1 \approx 6$ GHz. To record single-molecule spectra we used laser wavelengths about 0.2 nm from the inhomogeneous line centre for the O₁ 0–0 site origin at 592.321 nm (ref. 6).

Figure 1 shows excitation spectra taken at a series of laser focal spot positions. The three largest peaks isolated in both position and frequency are single pentacene defects. The centre frequency for these peaks does not change with time, and we denote this kind of defect behaviour as class I. The width of the peaks along the position axis (FWHM = 5 μ m) is a direct measure of the intensity profile of the focused laser beam in the crystal. The width of the peaks in frequency varies from 8 to 13 MHz as the laser intensity at the defect varies with position. This broadening is due to saturation of the defect optical transition. Calculating this saturation intensity using a scheme of three energy levels⁹ gives an estimated intensity of 1.8 ± 0.8 mW cm⁻², which is a factor of 40 lower than the value of 70 mW cm⁻² that one would calculate from pentacene photophysical parameters, including intersystem crossing¹⁰. Apparently (and not too surprisingly), the local pentacene environment this far out in the wings of the line produces alterations in the intersystem crossing rates. In addition to the class I peaks, there are smaller features in Fig. 1 that do not follow a definite trend with position, suggesting a time dependence from scan to scan. We denote defects that give time-dependent peaks as class II.

Figure 2 shows a typical time sequence of excitation spectra obtained at a fixed laser spot position. The spectra contain three time-independent class I peaks, which form stable ridges along the time axis near -130, -70 and +10 MHz. In the region 0–150 MHz is a single class II defect whose frequency varies from one scan to the next. Indeed, this same molecule can appear several times in the same 2-min laser scan, or almost not at all. Note that in the same volume of crystal (~ 50 μ m³) and

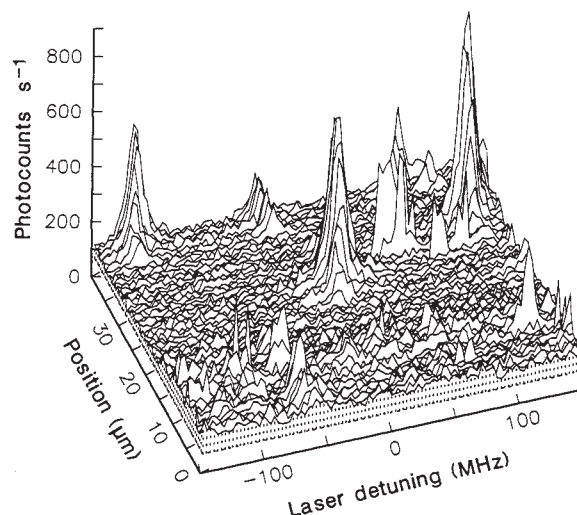


FIG. 1 Spectral 'landscape' for fluorescence excitation of a single pentacene defect in a crystal of *p*-terphenyl. 0 MHz detuning = 592.544 nm, $T = 1.5$ K, laser power = 1.5 nW, 2 min per spectrum.

at nearly the same frequency there are both class I defects and spectrally migrating class II defects.

If the laser frequency in Fig. 2 is fixed at any detuning from 0 to +100 MHz, the collected fluorescence turns on and off discontinuously like a random telegraph¹¹ or quantum jump¹² signal. The absence of fluorescence, however, means only that the defect is not absorbing at the laser frequency. To follow the resonance frequency directly, we scanned more rapidly and sacrificed optimal signal-to-noise ratio in order to measure the spectral position of the defect once each second. Figure 3a shows how the resonance frequency of a spectrally jumping class II defect evolves with time. A computer locates the peak in each scan and records this resonance frequency as a function of time in Fig. 3b. The optical transition energy seems to have a preferred set of values and performs spectral jumps between these values that are discontinuous on the 1-s timescale of the measurement.

Using several samples from the same sublimation run, a variety of class II defects have been observed that have two or more preferred frequencies spaced by 20–60 MHz with mean times between jumps of 1–420 s. Moreover, the occurrence of class II behaviour is quite common in the wings of the inhomogeneous line (to the red as well as to the blue). By observing 133 single molecules at several positions towards the red from O₁, the fraction of class II defects increases from 13% at 592.365 nm to 40% at 592.550 nm. On the other hand, using a thin, strain-free, lightly doped sample with an impurity concentration of 5×10^{-9} mol mol⁻¹ and $\Gamma_1 = 2$ GHz, single molecules could be observed 0.006 nm from the line centre and only class I behaviour was found. We conclude that class II behaviour is related to the improbable, highly strained local environments that occur far out in the wings of the inhomogeneous line.

The mean time between spectral jumps varies by no more than 20% for laser intensities from 3.3 to 65 mW cm⁻². Therefore,

the spontaneous spectral jumps do not seem to be photo-induced changes such as that which occurs when a persistent spectral hole is formed near the centre of the inhomogeneous band¹³. By analogy with glasses, we call the spectral jumping effect spectral diffusion, because the absorbing molecule seems to be coupled to an ensemble of diagonal (energy-changing) perturbations that shift the resonance frequency in a stochastic manner. On the other hand, class I defects have not been observed to jump with the laser frequency fixed at the resonance frequency for periods of up to 10 min, or by scanning and detecting the resonance at regular intervals of up to 55 min. Thus we suggest that class I defects are isolated pentacene molecules that have stable surroundings.

Measurements of the temperature dependence and the power-broadening behaviour for class I and class II molecules show that both the saturation intensity and dephasing rates differ from centre to centre and from class to class. All centres seem, however, to have the same low-power lifetime-limited linewidth $\gamma \approx 7.8 \pm 0.2$ MHz, in agreement with coherent transient data⁵. By comparison with the measured properties of dimers of pentacene in *p*-terphenyl¹⁴, we find no strong evidence that either class of defect represents dimers. Individual defects at nearly the same frequency far in the wings of the inhomogeneous line each have unique spectral-jumping behaviour, which suggests different nearby host configurations. We are thus led to propose that class II defects are located in regions of the crystal where a variety of local structural configurations are energetically accessible at low temperatures. These local configurations may be modelled by an ensemble of two-energy-level systems, by analogy with the similar problem for glasses¹⁵. Phonon-assisted tunnelling¹⁶ can occur between the two energy levels, and the pentacene resonance shifts with changes in the quantum numbers that describe the nearby host crystal configuration. One

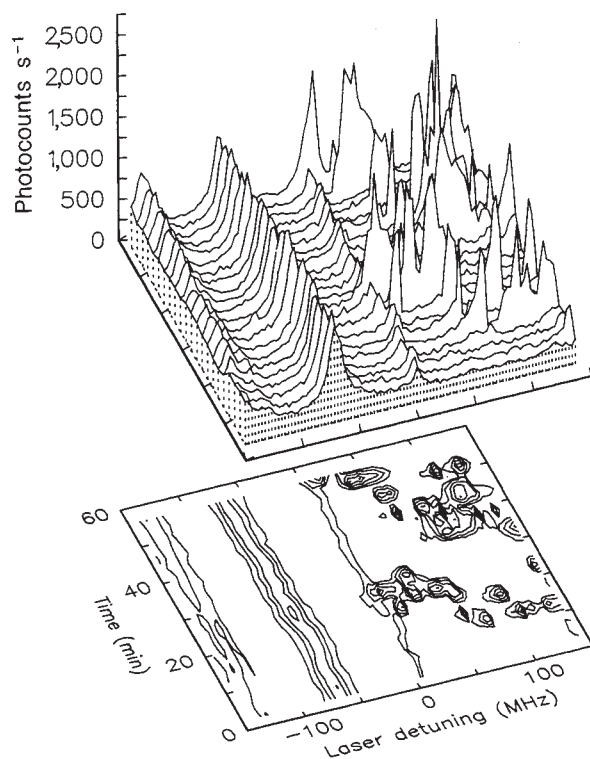


FIG. 2 Time dependence of fluorescence excitation spectra of a single defect. Top, 'wire' plot. Bottom, contour plot. 0 MHz detuning = 592.539 nm, laser power = 1.5 nW, 2 min per spectrum. The appearance of class I behaviour at negative detunings and class II behaviour at positive detunings has no significance.

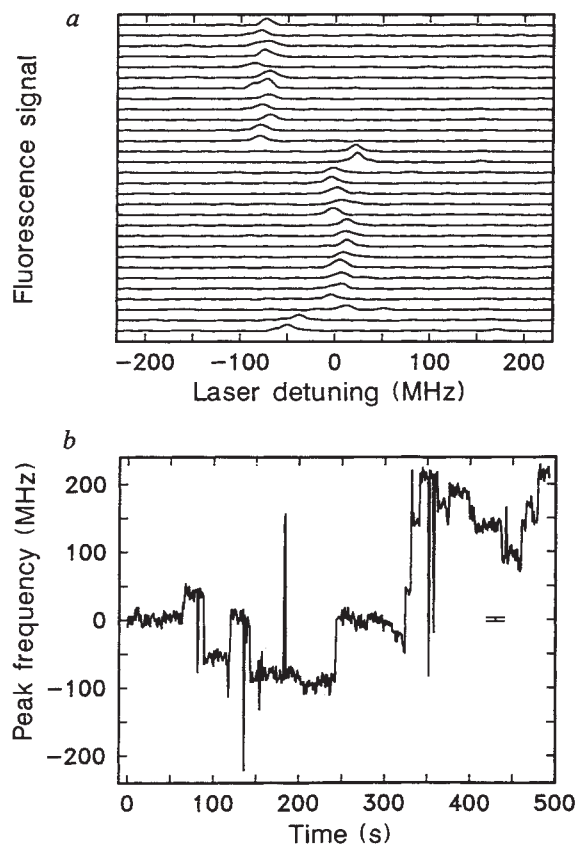


FIG. 3 Spectral jumps in the resonance frequency of a class II single pentacene defect. a, Individual 1-s spectra. b, Time dependence of centre frequency; 0 MHz detuning = 592.546 nm.

possible source for the two-level systems would be tipping of the central phenyl ring of *p*-terphenyl between various allowed orientations that are made accessible by disorder in the crystal structure¹⁷. Validation of this model requires detailed temperature-dependent measurements of the jump rate for a single centre over a wide range of temperature in hope of observing the characteristic $\coth(E/2kT)$ dependence, where E is the energy splitting in an asymmetric two-level model and k is Boltzmann's constant.

As the spectral jump rates and sizes that we observe are different for different defects, direct observation of this spectral diffusion effect would not be possible in any spectroscopy that averages over many defects. Direct measurement of the behaviour of individual guest molecules in a glass would lead to a clearer understanding of the origins of dynamics in amorphous solids^{18,19} than is available from ensemble-averaged measurements. Much more intriguing is the fact that we have seen spectral jumping in a crystalline environment, where such an effect is not expected. □

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Dynamical limitations on the Antarctic iron fertilization strategy

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MARTIN *et al.* have proposed an ingenious means by which the rise in atmospheric CO₂ content generated by the burning of fossil fuels and deforestation might be partially compensated¹. The idea is that plant production in the nutrient-rich surface waters of the Antarctic could be stimulated by the addition of dissolved iron, thereby reducing the CO₂ partial pressure in these waters and allowing CO₂ to flow from the atmosphere into the Antarctic Ocean. We have used a box model calibrated with transient tracer data to examine the dynamical aspects of this proposal, and conclude that after 100 years of totally successful fertilization the CO₂ content of the atmosphere would be lowered by only 10 ± 5% below what it would have been in the absence of fertilization. So if after 100 years the CO₂ content of the atmosphere were 500 μatm without fertilization, it would be between 425 and 475 μatm with full fertilization. In other words, if our model calibration is correct, even if iron fertilization worked perfectly it would not significantly reduce the atmospheric CO₂ content.

The CO₂ partial pressure in surface ocean water is influenced by the extent to which plant growth reduces the total CO₂ content, ΣCO₂, of surface water. The magnitude of this reduction depends on the efficiency with which the limiting nutrients phosphate and nitrate are utilized. In today's temperate and tropical ocean the utilization efficiency is high and therefore the reduction of the CO₂ partial pressure, p_{CO_2} , is near maximum. By contrast, the utilization efficiency is low in the polar oceans. Hence, were a means to be found to increase the efficiency of nutrient utilization in these waters, their CO₂ partial pressure and in turn that for the atmosphere, could be reduced. Martin and his co-workers²⁻⁶ have shown in incubation experiments that plant growth rates in waters from polar regions can be greatly accelerated by adding trace amounts of dissolved iron. Iron is an essential micronutrient required for the metabolism of all forms of life, its primary function being cytochrome formation. Because iron is one of the most particle-reactive elements, its concentration in the sea is very low, with the lowest values occurring in regions like the Antarctic that are most removed from the continents.

To date, most of the attention regarding the Martin proposal has been focused on its biological aspects¹ and little attention has been given to the dynamical aspects. Even though successful iron fertilization would initially produce up to a twofold decrease in the CO₂ partial pressure in Antarctic surface waters, this reduction might be short-lived. In the absence of water circulation, only an amount of CO₂ equal to that removed as a result of the initial iron fertilization would be sequestered from the atmosphere. In this case, the atmosphere, the Antarctic surface ocean and the non-Antarctic surface ocean would

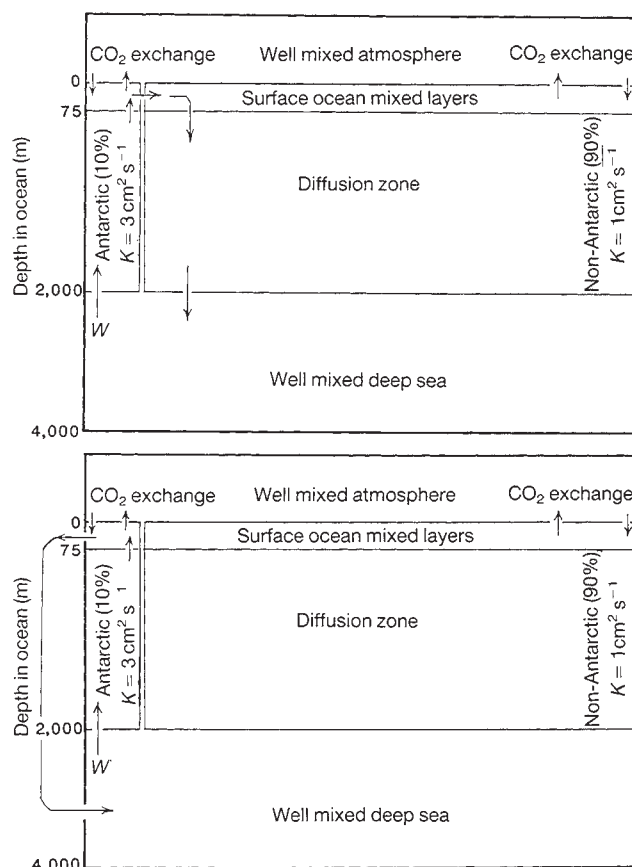


FIG. 1 Linked vertical advection and diffusion model used to evaluate the response to iron fertilization of Antarctic surface waters. The upper panel shows the case where the water upwelled in the Antarctic is transferred laterally to the non-Antarctic surface ocean. The lower panel shows the case where this upwelled water is converted to deep water and transferred directly to the model's deep reservoir.

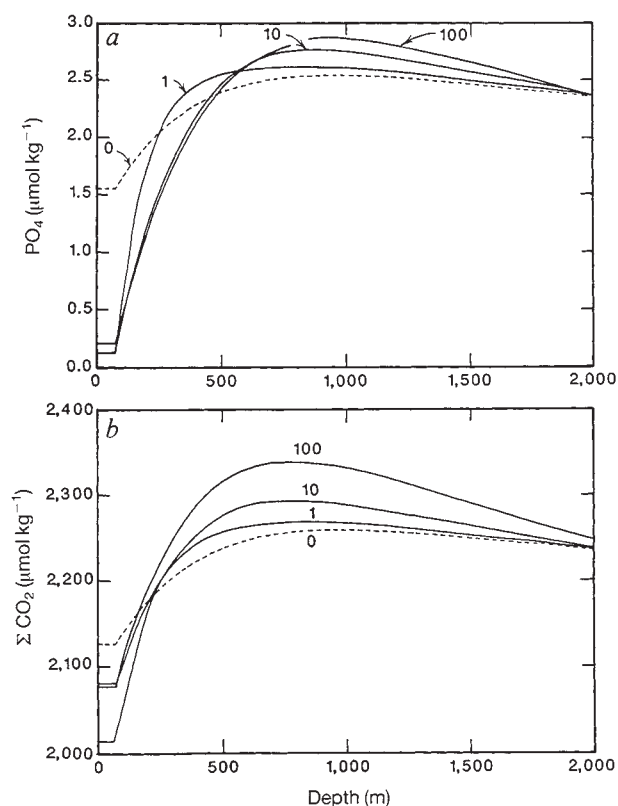


FIG. 2 Vertical distribution of *a*, PO_4 and *b*, ΣCO_2 in the Antarctic column before the onset of fertilization (dashed line) and 1, 10 and 100 years after the onset of fertilization for the scenario where water is upwelled at the rate of 17.4 Sv and transferred to the deep sea.

rapidly reach a new equilibrium, leaving the atmosphere with a CO_2 content only slightly lower than it had before fertilization. To achieve a significant reduction of the atmosphere's CO_2 content the surface waters of the Antarctic must be replaced frequently from below.

Thus the critical issue is the rate of vertical mixing in the Antarctic Ocean. Traditional thinking is that deep ocean water upwells in this region⁷. A portion of this upwelled water moves beneath the Antarctic's sea-ice fringe where it is densified by brine release causing it to sink back into the deep sea. The remainder moves to the north. Part is converted to intermediate water which penetrates northward into the Atlantic, Pacific and Indian oceans at a depth of $\sim 1,000$ m. The remainder is mixed into the temperate surface water.

Confirmation of the upwelling hypothesis comes from the inventories of bomb-produced radiocarbon in the Antarctic

Ocean as measured during the GEOSECS surveys of the world ocean⁸⁻¹⁰. These results show that the Antarctic waters contain far less bomb-produced radiocarbon than invaded this region from the atmosphere¹¹. By contrast, the temperate region of the Southern Hemisphere ocean has an inventory exceeding the amount received by invasion. This excess roughly balances the deficiency in the Antarctic. This pattern can only be explained by upwelling in the Antarctic coupled with lateral transport to the adjacent temperate regions¹¹. The upwelling rate required to explain the Antarctic deficiency is 15 yr^{-1} (ref. 11). As the low bomb-radiocarbon water in the Antarctic Ocean covers $\sim 10\%$ of the total ocean area, this corresponds to a flux of 17 Sv ($17 \times 10^6 \text{ m}^3 \text{ s}^{-1}$).

In addition to upwelling, account must be taken of the extent of vertical mixing. The mean penetration depth of the bomb radiocarbon for the fifteen GEOSECS radiocarbon profiles for latitudes south of 49°S is 260 m (ref. 11). This extent of penetration into the upwelling plume can be explained¹¹ using a vertical eddy diffusivity of $\sim 3 \text{ cm}^2 \text{ s}^{-1}$ (the steady-state tracer penetration depth into such a plume would be 630 m). Hence, if this interpretation of the bomb-radiocarbon data is correct, the volume of water immediately beneath the Antarctic surface available for CO_2 storage is only $2.2 \times 10^{16} \text{ m}^3$ ($630 \text{ m} \times 3.5 \times 10^{13} \text{ m}^2$) or 1.8% of the ocean's volume, a volume too small to house a significant amount of excess CO_2 . Thus it is the fate of the upwelled water that matters. Additional storage capacity will be provided by that portion of the upwelling water that is converted to bottom water along the southern fringe of the Antarctic or to intermediate waters along the northern fringe of the Antarctic.

We devised a simple model to test our ideas. Two box-diffusion columns¹² are linked by an overlying atmosphere and an underlying deep sea (see Fig. 1). One column represents the Antarctic and the other the non-Antarctic region of the ocean. Each column is capped by a mixed layer 75 m deep. These mixed layers are underlain by diffusion zones 2,000 m thick. Beneath the diffusion zone is a single well mixed deep reservoir. The area of the Antarctic column was taken to be 10% of the ocean total. The actual effective area of nutrient-rich water in the Antarctic is 16% of the ocean area (out to 45°S). We adopted a somewhat lower area to compensate for the fact that iron fertilization cannot work during the lightless winter months. During these periods vertical mixing will return the CO_2 partial pressure to its pre-fertilization value.

We set the vertical eddy diffusivity in the Antarctic column at $3 \text{ cm}^2 \text{ s}^{-1}$ and that in the non-Antarctic column at $1 \text{ cm}^2 \text{ s}^{-1}$. The upwelling rate in the Antarctic column was set at 17 Sv (15 yr^{-1}). Because we do not have information with which to apportion the fate of the upwelled water, we studied two limiting cases. In the first the water is transferred entirely to the surface of the non-Antarctic column. In the second it is transferred entirely to the deep reservoir.

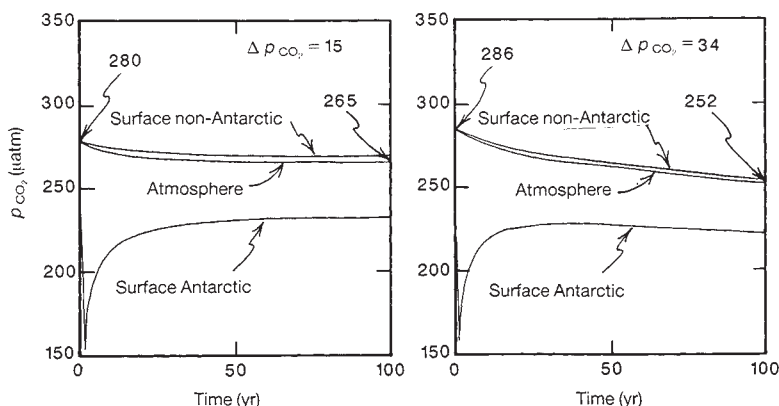


FIG. 3 Model runs for an Antarctic upwelling flux of 17.4 Sv. Left-hand panel shows the case where the upwelled water is transferred to the surface of the non-Antarctic Ocean and the right-hand panel shows the case where the upwelled water is transferred to the deep sea. In each case totally successful iron fertilization is conducted for 100 years. For the steady-state conditions before fertilization, the p_{CO_2} for Antarctic surface waters is nearly identical to that for the atmosphere.

We started our calculation with the carbon cycle in a steady state. The residence times for PO_4 with respect to biological removal from the surface reservoirs were set to yield $1.6 \mu\text{mol per kg PO}_4$ in the surface water above the Antarctic column and no PO_4 in the surface waters of the non-Antarctic column. The regeneration function for falling organic debris was set to yield PO_4 depth profiles similar to those observed (the choice of this regeneration function has no influence on the result of the calculation of the atmospheric CO_2 response to iron fertilization). The atom ratio of carbon to phosphorus in the organic matter falling from the surface mixed layer was 130. We then adjusted the $\Sigma\text{CO}_2/\text{alkalinity}$ ratio in the model ocean to yield an atmospheric p_{CO_2} of $280 \mu\text{atm}$.

We simulated a totally successful iron fertilization by greatly decreasing the residence time (with respect to biological removal) of PO_4 in Antarctic surface water, bringing its PO_4 content to near zero, and continued the iron fertilization for 100 years. Figure 2 shows the evolution of the vertical distributions of PO_4 and ΣCO_2 in the Antarctic column for the deep-transport case. The total PO_4 in the water column remains unchanged but a bulge of excess ΣCO_2 appears, representing the CO_2 transferred from the atmosphere to the Antarctic water column. In the lateral-transfer scenario (see Fig. 3), the atmospheric CO_2 content drops ever more slowly, reaching an asymptote $\sim 15 \mu\text{atm}$ lower than the initial value. In the deep-transfer scenario the decrease continues, reaching $\sim 34 \mu\text{atm}$ after 100 years. The reason for the difference is that in one case the surface water from the Antarctic is transferred to the surface of the non-Antarctic region, allowing the excess CO_2 to re-enter the atmosphere, whereas in the other this water is removed to the deep sea, isolating it from the atmosphere.

As a sensitivity test we have changed the upwelling rate for the deep-transfer case. A rate of 8.7 Sv yields a drop in atmospheric p_{CO_2} of $28 \mu\text{atm}$ and that of 34.8 Sv a drop of $47 \mu\text{atm}$. The question naturally arises as to what would happen if iron fertilization were terminated. Our model shows that any reduction in atmospheric CO_2 content accomplished by the fertilization would be lost, on more or less the same timescale as it was gained.

Clearly, the key to the evaluation of the iron-fertilization scheme is the rate of vertical overturn in the Antarctic. If our interpretation of the bomb-radiocarbon record is correct, then this process is too slow to create a significant decrease in the CO_2 content of the atmosphere. The distribution of freons in the Antarctic water column offers further evidence in this regard. The single published freon section¹³, a meridional traverse across the Atlantic sector of the Antarctic, shows that the freon concentration for the entire water column between 500 m below the surface and 500 m above the bottom is $<6\%$ of the surface concentration. Although we have not formally modelled freons, this result is broadly consistent with the dynamics adopted here. In any case, such surveys offer constraints on the rates of both deep- and intermediate-water formation. As such information is critical to the evaluation of temperature buffering of the greenhouse warming by the Antarctic Ocean¹⁴, as well as to evaluation of the iron fertilization scheme, its procurement should be placed high on the list of environmental research priorities. □

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ACKNOWLEDGEMENTS. Discussions with J. Sarmiento prompted this study. He also pointed out an important mistake in our paper. We had assumed that the reductions obtained for a steady-state atmosphere with 280 p.p.m. CO_2 applied directly to the anthropogenically perturbed atmosphere. Sarmiento pointed out that this is not correct. We have carried out CO_2 -increase models and found that the percentage change in CO_2 partial pressure and not the absolute change that remains approximately the same. We have done the perturbation calculations and find that this is indeed correct. This work was supported by the Lawrence Livermore National Laboratory, Exxon Corporation and the Carbon Dioxide Research Program (W.S.B.) and by the U.S. Department of Energy (T.H.P.) through a joint contract Martin Marietta Energy Systems Inc.

Control of pore-water chemistry at the base of the Florida escarpment by processes within the platform

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PORE waters collected from seep sediments hosting active chemosynthetic communities^{1,2} tend to be rich in sulphide, chloride and ammonium and depleted in sulphate relative to the concentrations in sea water. To investigate the source of the energy-rich compounds and the processes causing low sulphate concentrations in seep-sediment pore waters, we have measured the sulphur isotope composition, $\delta^{34}\text{S}$, of pore waters from seep sediments at the base of the West Florida escarpment. The isotopic composition of pore-water sulphate remains approximately constant as its concentration is depleted, indicating that processes within the Florida platform, rather than microbial processes at seep sites, control pore-water chemistry in these sediments. The composition of the seep brines, deduced from a linear mixing model, provides information on processes deep within the platform.

Dense biological communities living on highly sulphidized sediments found at the 3,280-m-deep base of the Florida escarpment are supported by chemosynthetic processes^{1,3,4} that use reduced substrates seeping from the highly jointed limestone cliff forming the edge of the Florida platform^{2,5} (Fig. 1). These seeps occur along at least 10% of the escarpment base⁶. Sedimentary pore waters beneath the large populations of mussels and tube worms are enriched in ammonium (up to 2 mM)⁷, radiocarbon-depleted biogenic methane (up to 10 mM)^{5,8} and sulphide (up to 5.7 mM). Sediments consisting of $>30\%$ sulphide minerals have been reported⁹. The acidity generated by sea-floor oxidation of dissolved sulphide has been suggested as one mechanism for undercutting and eroding the edge of the carbonate continental margin².

We collected sediment cores in these chemosynthetic communities with the submersible *Alvin*. The pore waters were expressed from sediments by pressure filtration. Depth profiles of pore-water concentrations show chloride and ammonium enrichment and corresponding sulphate depletion relative to overlying sea water (Fig. 2). Both pore-water sulphate and ammonium are linearly correlated with pore-water chloride concentrations (Fig. 3), indicating conservative mixing between sea water and a sulphate-depleted, ammonium-rich brine. The chloride-sulphate trend yields a value of 27.5% for a sulphate concentration of zero, suggesting that this is the chlorinity of the brine exiting the platform at the sea floor. The equation fitting the chloride-ammonium data yields a value of 2.2 mM for the concentration of ammonium in the sulphate-depleted brine.

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The sulphur isotopic composition of dissolved sulphate in seep-sediment pore waters does not differ from the isotopic composition of sulphate in the overlying sea water (Fig. 4). Sulphate depletion in marine sediments, however, is generally controlled by bacterial decomposition of recently deposited organic matter¹⁰ following the depletion of oxygen. Preferential bacterial use of ^{32}S sulphate typically leaves the residual sulphate heavier than the $\delta^{34}\text{S}$ of 20.4‰ found in sea water¹¹ (Fig. 4). Thus, local biological sulphate reduction must not occur at an appreciable rate relative to advective removal of sulphate in the seep sediments where these cores were collected. Precipitation of sulphate minerals could also cause the observed sulphate depletions and would be consistent with the sulphur isotope data, but inorganic precipitation would not produce the observed simultaneous linear changes in the chloride and ammonium concentrations. Thus the sulphur isotope data support our hypothesis that conservative mixing between normal sea water and a sulphate-depleted brine produces the chemical distributions observed in seep-sediment pore waters.

Measurements of chloride, calcium and sodium concentrations in seep pore waters, strontium isotope ratios, and the fluid oxygen and hydrogen isotopic composition confirm that the seep brine is a mixture of ~6% platform-formation brines^{2,12} containing up to 250 g kg^{-1} dissolved solids¹³⁻¹⁵ and 94% sea water, which enters the platform along its exposed flanks (Fig. 1). Pore-water $\delta^{18}\text{O}$ and δD (-0.3% and $+2\%$, respectively, at 26.3‰ chlorinity¹²) do not indicate the admixture of meteoric waters from the Floridan aquifer, which is separated from the deeper saline aquifer at a depth of ~1 km by the relatively impermeable anhydrite of the Cedar Key formation^{14,15}. The sulphide arriving at the seeps must be derived from the mixing of seawater sulphate into the formation brine within the platform through lateral flow^{2,9} and from dissolving evaporite sulphate deposits in the platform (13–23%)^{16,17}.

The lack of sulphate in seep brines indicates that all sulphate in this mixture must be quantitatively reduced to sulphide during migration through the platform before exiting at the escarpment's base. Sulphate concentrations in the formation brine are less than the seawater value of 29 mM; the sulphide concentrations are unknown¹³. Because microbial sulphate reduction produces sulphides depleted in $\delta^{34}\text{S}$, it was previously hypothesized^{2,9} that the similarity between the $\delta^{34}\text{S}$ values of sulphide compounds at the seep and those in the platform evaporite

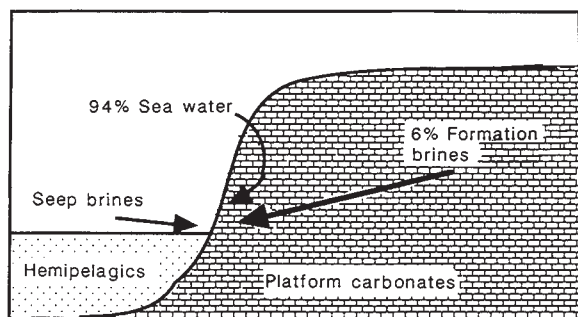


FIG. 1 Schematic east-west cross-section of the Florida platform at about 26°N . The composition of fluids exiting the platform and mixing with sea water at seeps at the escarpment base is controlled by inner-platform mixing of dense formation brines from the platform centre with sea water entering at the platform's flanks. This circulation reduces the geothermal gradient in the flanks. Migration through strata containing organic matter subsequently converts sulphate quantitatively to sulphide, adds ammonium, and finally methane. The formation's brine composition is ¹³: Cl, 4,287 mM (148.3‰); Na, 2,475 mM; Mg, 4,770 mM; SO_4 , 1.4 mM; Ca, 692 mM; Sr, 13.1 mM. The composition of the seep brine is ¹²: Cl, 797 mM (27.5‰); Na, 599 mM; Mg, 74.9 mM; SO_4 , 0.0 mM; Ca, 43.2 mM; K, 104 mM; Sr, 3.2 mM; I, 0.067 mM.

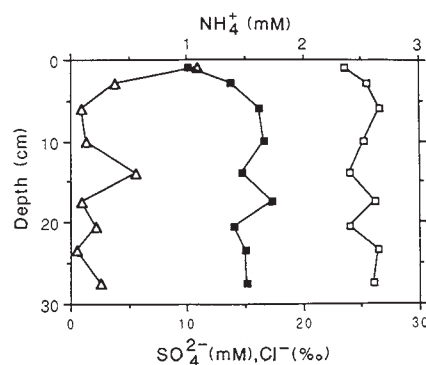


FIG. 2 Concentration of Cl^- ($\square$), SO_4^{2-} ($\triangle$) and NH_4^+ ($\blacksquare$) measured in ALVIN push core 1765-4B. Overlying sea water has ~19‰ Cl^- , 29 mM SO_4^{2-} , and 0 mM NH_4^+ . This core was obtained at $26^\circ 05' \text{N}$, $84^\circ 55' \text{W}$, ~2 m from the base of the escarpment; collected near a small fan-shaped seep, this core sampled the 'front' of the advancing fluids. Organic carbon varied unsystematically with depth, from 0.8 to 1% by weight.

deposits suggested thermally mediated sulphate reduction, a process that does not produce sulphur isotope fractionation¹⁸⁻²⁰. But because the observed quantitative conversion of sulphate to sulphide should produce no net isotope fractionation, the possibility of microbially mediated sulphate reduction cannot be eliminated from isotope considerations alone, and is also consistent with the 9–20‰ $\delta^{34}\text{S}$ measured in the advecting sulphide^{2,7,9}.

Sulphate reduction within the platform could be a two-stage process, with sulphide generated thermochemically in formation brine at the peninsula's centre and with seawater sulphate reduced microbially at the platform's cooler edge. The lower temperature limit for thermochemical sulphate reduction and the upper limit for biogenic sulphate reduction are in the ranges 80–200 °C and 80–100 °C, respectively¹⁸⁻²³. Temperature regimes suitable for both processes exist within the platform. From elevated temperatures near the peninsula's centre (114 °C), isotherms bend downward at all depths (from 0.5 to 6 km) near the platform's edges owing to lateral heat and seawater flow^{14,15,24}. Kohout reports temperatures as low as 73 °C at depths of 4.6 km near the platform's edge^{14,15}, consistent with the low ambient temperatures measured within seep sediments and the presence of fossil biogenic methane^{5,8}. Pore-fluid oxygen isotope ratios (given above) are indistinguishable from seawater values, and do not indicate any isotope exchange with carbonate rocks. Isotope exchange between water and calcite begins to occur at temperatures $> 70^\circ\text{C}$ (ref. 25).

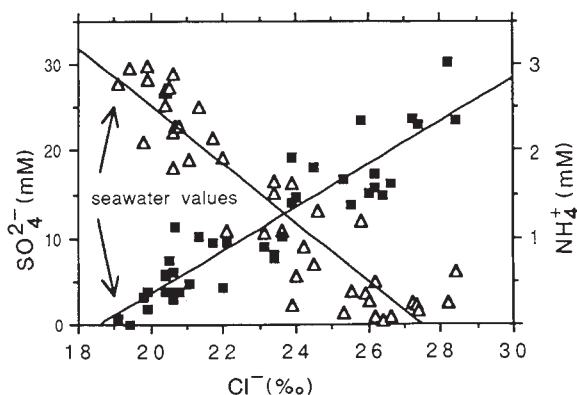


FIG. 3 Concentration of pore-water SO_4^{2-} ($\triangle$) and NH_4^+ ($\blacksquare$) plotted against Cl^- for seep cores collected on ALVIN dives 1762–1765, 1769 and 1770 ($r = 0.92$, $n = 43$ for $\text{Cl}-\text{SO}_4^{2-}$; $r = 0.92$, $n = 39$ for $\text{Cl}-\text{NH}_4^+$). Similar regressions for individual cores yield r values closer to 1.0.

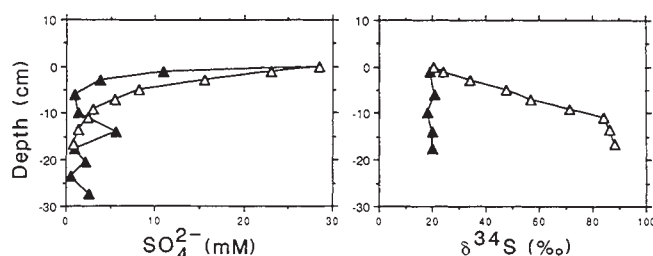


FIG. 4 *a*, Pore-water sulphate concentrations and *b*, $\delta^{34}\text{S}$ plotted against depth for Florida escarpment seep core 1765-4B ($\blacktriangle$) and for a core typical of marine sediments ($\triangle$) dominated by microbial sulphate reduction³⁴ (Cape Lookout bight, North Carolina, USA). $\delta^{34}\text{S}$ does not change in seep profiles as sulphate concentrations decrease, in stark contrast to typical marine sediments where values become enriched in the heavy isotope owing to fractionation by sulphate-reducing bacteria. Isotope profiles identical to data from core 1765-4B were obtained from three other cores at separate locations. Values at 0 cm depth were obtained from measurements of overlying sea water. $\delta^{34}\text{S} = ((^{34}\text{S}/^{32}\text{S})_{\text{sample}} / (^{34}\text{S}/^{32}\text{S})_{\text{standard}} - 1) \times 1,000$, where the standard is CDT.

Thermogenic methane has been observed in association with thermochemical sulphate reduction²⁶, but the abundant methane advected into Florida escarpment seep sites is clearly of biogenic origin ($\delta^{13}\text{C}$ from -61 to -94%)⁸. Accelerator ^{14}C measurements suggest a largely fossil-carbon source for this methane⁵, indicating a source of ancient metabolizable organic matter upstream of the seeps. Sufficient organic matter is present below the Cedar Key formation to generate petroleum deposits in geothermally hotter southern Florida^{24,27-29}, and may be associated with carbonate rocks³⁰ or evaporite deposits³¹. In contrast, rates of sulphate reduction in migrating fluids within the surficial Floridan aquifer are limited by organic matter. Sulphate is not depleted, and sulphide exhibits negative $\delta^{34}\text{S}$ values, typical of biogenic sulphide in the presence of sulphate¹⁷.

Sulphate depletion is critical for two processes occurring within the platform. Methanogenesis proceeds in the absence of sulphate³², which suggests a mechanism for generating the observed large quantities of biogenic methane by microbial organic carbon remineralization downstream of the sulphate consumption. Sulphate depletion also facilitates the formation of dolomite³³, which occurs within the platform^{27,28}.

Abyssal seeps provide information about the nature of the sub-surface processes that generate fluids containing large quantities of dissolved reduced compounds. The chloride composition of the fluids exiting the Florida platform is determined by mixing of sea water with dense formation brines within the platform². As the combined fluid migrates through strata containing organic matter, sulphate is quantitatively reduced to form the abundant ^{34}S -rich sulphides which are ultimately deposited at the escarpment base. Downstream of sulphate exhaustion, biogenic methane is produced⁸. The ammonium is generated throughout the platform by the decomposition of organic matter^{1,2}. At the escarpment base, these energy-rich fluids exit the platform, contact oxygen-rich sea water, and fuel chemosynthetic food chains. $\square$

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Effect of fluorine on solid-state alkali interdiffusion rates in feldspar

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SOLID-STATE diffusion plays an important part in controlling the chemical and physical nature of minerals and rocks, and the mechanism and kinetics of diffusion are in turn affected by the physical and chemical environment. We have investigated the effects of fluorine on alkali interdiffusion in feldspars because it is known that fluorine strongly affects the structure and viscosity of melts¹, fluid-melt partition coefficients², mineral melting temperatures^{3,4} and, most significantly, the rate of cation diffusion in melts⁵. We report the results of diffusion-couple experiments run at 600-800 °C and 200 MPa for 2-24 days. At relatively low fluorine fugacities the alkali interdiffusion rate was increased by about four orders of magnitude compared with the fluorine-absent system, and the activation energy was decreased. The magnitude of the effect is great enough to explain unexpectedly large exsolution lamellae in some rhyolites, and also calls into question the effects of fluorine on other solid-state reactions involving diffusion. Because fluorine is a common volatile component in igneous and metamorphic rocks, the implications of our results may extend far beyond this simple example.

We measured the Na-K interdiffusion rate $\bar{D}$ using diffusion-couple experiments. Single crystals of Amelia albite (1.1% orthoclase, 98.6% albite, 0.3% anorthite, $\text{Or}_{1.1}\text{Ab}_{98.6}\text{An}_{0.3}$) and Kristalina adularia ($\text{Or}_{89.6}\text{Ab}_{10.1}\text{An}_{0.3}$) were cored perpendicular to the (010) face and the face polished with 0.25- μm diamond paste. The crystals were bound tightly together using either a tight gold tube or platinum wire, and were sealed dry in a noble metal tube with either a nickel/nickel oxide or haematite/magnetite (calcite-graphite-fluorite) (NNO(CGF) or HM(CGF)) fluorine buffer (Fig. 1)



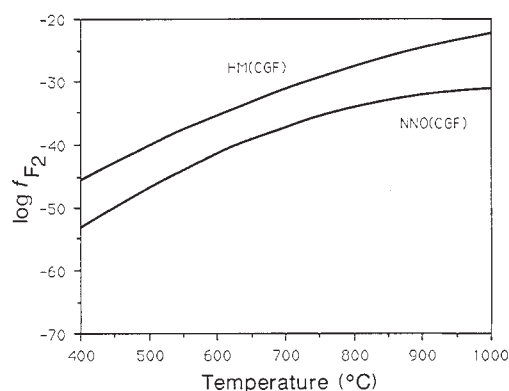


FIG. 1 Log fluorine fugacity (in bars) plotted against temperature for the two buffers used, calculated from equations (1) and (2), at 200 MPa.

X-ray diffraction following the experiments confirmed that all components of the buffers were still present. To keep the experimental charges dry we stored the buffers in a sealed oven at 110 °C. The crystals and the noble-metal tubing were also stored in the oven after preparation and before assembly, for at least a few hours. Relative humidity in the laboratory was generally <20%, so that absorption of water during assembly was not a problem. These precautions are important because feldspar dissolves easily in HF solution. There are no experimental data on the fluid-to-solid ratio needed for significant dissolution, not are we aware of studies of the dissolution of minerals in a nominally dry fluorine atmosphere. We sought, but did not find, evidence for dissolution in our experimental products. At this low fugacity, fluorine is apparently soluble in feldspar, rather than the other way around.

Experiments were run in hydrothermal bombs at 200 MPa, and temperatures from 600 to 800 °C for 2–24 days (at temperatures ≥ 850 °C melting occurs because fluorine lowers the melting point of feldspar). Afterwards, the capsules were weighed to confirm that the seals had held, then sliced and polished for microprobe analysis. Composition profiles (Fig. 2) were measured with a Cameca SX50 electron microprobe, using a step size and a spot size of 0.5 μm . The data were smoothed by eye, and the resulting profile (Fig. 2) and the standard Wagner equation⁶ used to calculate the interdiffusion coefficient as a function of composition. Smoothing was generally not necessary toward the centres of the profiles, but was used near the ends because the slope of the profile (which appears in the Wagner equation) must always be positive. Some longer experiments, such as the one at 600 °C (Fig. 2), show a homogeneous mixing zone in the albite up to 10 μm wide with a composition of about Or_{5-7} . The adularia side of the diffusion couple never shows this effect. One possible explanation is that the composition Or_{5-7} is the preferred stable composition at these conditions. Because it is so close to the starting composition of the albite, this should have little effect on the diffusion rates. Owing to this anomaly, however, we consider $\bar{D}$ values determined for Or_{50-90} to be more accurate.

Figure 3 shows an Arrhenius plot of our data together with data from the experiments of Christoffersen *et al.*⁷ on Na–K interdiffusion in a non-fluorine atmosphere, all for the composition Or_{70} . A difference of about seven orders of magnitude in fluorine fugacity (Fig. 1) results in undetectable differences in $\bar{D}$, so that in Fig. 3 we show the data with both buffers. The estimated error, based on repeated experiments and analyses and uncertainties in the calculations, is about one or two orders of magnitude. The addition of fluorine increases the interdiffusion rates by three or four orders of magnitude and lowers the activation energy from ~ 260 to 180 kJ mol^{-1} .

The experiments shown for comparison⁷ also used a diffusion couple and crystals from the same localities we used. Experiments at 500–1,500 MPa (ref. 7) combined with those at 200 MPa (ref. 8) indicate that the activation volume for alkali interdiffusion is negligible; our data at 200 MPa are therefore directly comparable to those of Christoffersen *et al.*⁷ at 1,500 MPa. The most significant difference in the data sets, other than the fluorine, is the anisotropy of $\bar{D}$; it is slowest perpendicular to (010) and about 10 times faster perpendicular to (001) (ref. 7). The most complete data sets, which include the fast direction for non-fluorine experiments and our fluorine experiments measuring $\bar{D}$ in the slowest direction, are compared in Fig. 3. The difference in $\log \bar{D}$ shown here, about three or four orders of magnitude, would be even larger if the data sets were for diffusion in the same direction.

In the run products, the level of fluorine in the diffusion zone is very low, and drops to the background level outside. The microprobe resolution is not good enough to determine whether some fluorine penetrated beyond the diffusion zone, or whether the alkali interdiffusion is limited by the rate of fluorine diffusion. Fluorine self-diffusion rates have not been measured in crystals: we can estimate a minimum rate by assuming that the fluorine penetrates no farther than the diffusion zone. Estimates range from $\sim 5 \times 10^{-15}$ to $1 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1}$ for the temperature interval measured, about the same as the rate of oxygen self-diffusion in feldspars at these temperatures. In dry silicate melts, fluorine diffusion is about an order of magnitude faster than oxygen diffusion, and several orders of magnitude faster than cation diffusion^{5,9,10}. It is independent of the amount of fluorine from 0.1 to 6.3% (ref. 9), but is dependent on melt composition, because of melt polymerization^{1,9}.

One possible explanation for the observed increase in alkali interdiffusion rates in a fluorine atmosphere is that fluorine replaces oxygen in the crystal, inducing alkali vacancies and increasing alkali mobility. The fluorine ion (1.39 Å) is about the same size as oxygen (1.40 Å) and is much more reactive and electronegative. The substitution of F^- for O^{2-} , however, requires the loss of a cation to maintain charge balance. Extrinsic defects of this kind are known to increase diffusion rates in other materials¹¹. If fluorine is indeed replacing oxygen in the structure, the mechanisms of fluorine and oxygen self-diffusion might be the same; our estimate of similar rates is consistent with this model.

These data may help explain the unusually coarse cryptoperthite lamellae observed in the rhyolitic Bishop Tuff¹². Lamellar coarsening is largely controlled by the Na–K interdiffusion rate. Previously measured rates were much too slow

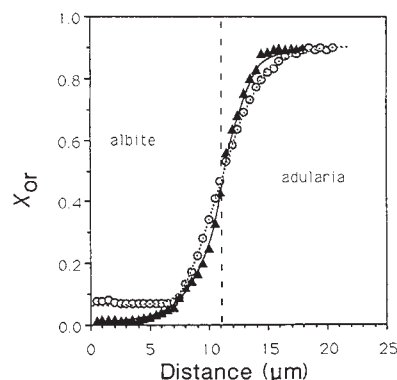


FIG. 2 Two interdiffusion profiles. Open circles, raw data for an experiment at 600 °C for 8 days; triangles, results from an experiment at 750 °C for 3 days. Lines through the data points are smoothed profiles used to calculate $\bar{D}$ as a function of composition. Vertical dashed line is the boundary between original crystals.

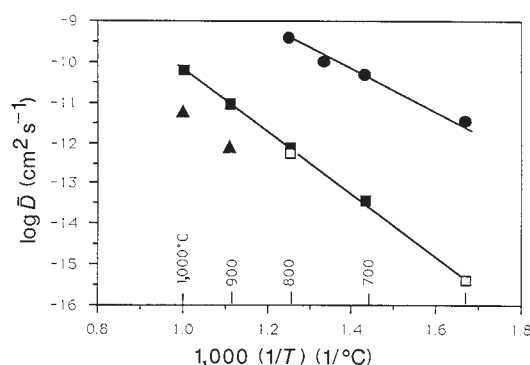


FIG. 3 Arrhenius plot comparing our data (●) for interdiffusion perpendicular to (010) with previous studies. ▲, experimental data⁷ for diffusion perpendicular to (001) at 1,500 MPa; ■, predicted⁷ values at 1,500 MPa; □, predicted³ values at 200 MPa. The difference in predicted and measured values is attributed to combined error in $\bar{D}$ and D^* , and the extrapolation of D^* values to higher temperature⁷. All data are for Or₇₀ composition.

to explain the observed spacings in sanidine crystals from this rhyolite, and the inferred presence of abundant fossil fumeroles suggested the possibility of a gas-phase component increasing the diffusion rates. The enhancement of interdiffusion rates by fluorine reported here is sufficient to account for the large

spacings. The geological implications, however, may go much farther. The fluorine fugacity in these experiments is five to ten orders of magnitude lower than that observed in fluorite-bearing metamorphic rocks¹³. As we observe no fugacity dependence over seven orders of magnitude, our experiments may be directly relevant to common rocks. In addition, the activation energy for interdiffusion in these fluorine-rich experiments is significantly lower than for those without fluorine, making the difference in $\bar{D}$ even greater at lower temperatures. Fluorine, even in small amounts, may strongly affect chemical zoning profiles, metamorphic growth rates and the development of microstructures. □

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Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry

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MICROBIAL activity in aquifers plays an important part in the chemical evolution of ground water^{1–5}. The most important terminal electron-accepting microbial processes in deeply buried anaerobic aquifers are iron reduction, sulphate reduction and methanogenesis^{5–8}, each of which requires simple organic compounds or hydrogen (H₂) as electron donors. Until now, the source of these compounds was unknown because the concentrations of dissolved organic carbon and sedimentary organic carbon in aquifers are extremely low^{9–11}. Here we show that rates of microbial fermentation exceed rates of respiration in organic-rich aquitards (low-permeability sediments stratigraphically adjacent to higher-permeability aquifer sediments), resulting in a net accumulation of simple organic acids in pore waters. In aquifers, however, respiration outpaces fermentation, resulting in a net consumption of organic acids. The concentration gradient that develops in response to these two processes drives a net diffusive flux of organic acids from aquitards to aquifers. Diffusion calculations demonstrate that rates of organic acid transport are sufficient to account for observed rates of microbial respiration in aquifers. This overall process effectively links the large pool of sedimentary organic carbon in aquitards to microbial respiration in aquifers, and is a principal mechanism driving groundwater chemistry changes in aquifers.

The chemical evolution of groundwater is characterized by increasing concentrations of dissolved constituents as water flows along hydrological gradients^{12,13}. Many of the most important dissolved constituents added to ground water, in terms of chemical reactivity and mass balance, are the result of microbial processes. For example, elevated concentrations of dissolved ferrous iron, hydrogen sulphide and methane in aquifers can

result from microbially mediated iron reduction, sulphate reduction and methanogenesis, respectively^{5–7}. Furthermore, the degradation products (such as CO₂ and organic acids) of these and other microbial processes react with aquifer sediments to cause elevated concentrations of dissolved inorganic carbon, sodium and silica in ground water^{11,14,15}. Here we study the mechanism by which microbes in deeply buried aquifers (20–350 m) of the Atlantic coastal plain are supplied the simple organic substrates needed to maintain their metabolism.

To determine the distribution of substrates in these sediments, we collected pore water in freshly cored, sulphate-reducing sediment^{16,17} from near Lake City, South Carolina and analysed it for volatile fatty acids (C₁–C₄) (Fig. 1). There were substantial amounts of formate and acetate in aquitards (55–110 μM), and lesser amounts in aquifers (30–35 μM). Owing to poor recovery of the most permeable unconsolidated sands during coring, pore water in these aquifer sediments was collected from a nearby municipal water-supply well. These samples contained only trace amounts of formate (3 μM) and acetate (<3 μM). The fact that the aquifer sediments are actively sulphate-reducing, whereas little respiratory activity was detected in aquitard sediments in this and previous studies^{17,18}, suggests that the low organic acid concentrations in aquifers are maintained by microbial respiration. As a result, a concentration gradient develops (Fig. 1), and a net diffusive flux of organic acids from aquitards into adjacent, microbially active aquifers is possible.

The diffusive fluxes of formate and acetate from the aquitards can be estimated using Fick's first law of diffusion [equation (1)], and the rate of microbial respiration in aquifers that can be maintained by these fluxes can be estimated from equation (2).

$$J_{f,a} = -D_{f,a} (dC_{f,a}/dz) \phi_c \quad (1)$$

$$R_{CO_2} = 2J_a/T\phi_s + J_f/T\phi_s \quad (2)$$

where $J_{f,a}$ is the flux of formate (f) or acetate (a) into aquifers (mmol ml⁻¹ yr⁻¹), $D_{f,a}$ is the diffusivity of formate (1.4×10^{-2} m² yr⁻¹)^{19,20} or acetate (8.7×10^{-3} m² yr⁻¹) in sediment, $dC_{f,a}/dz$ is the average concentration gradient of formate (0.0095 mmol l⁻¹ m⁻¹) or acetate (0.0080 mmol l⁻¹ m⁻¹) in Fig. 1, ϕ_c is the effective porosity of aquitard (clay) sediments (0.1)

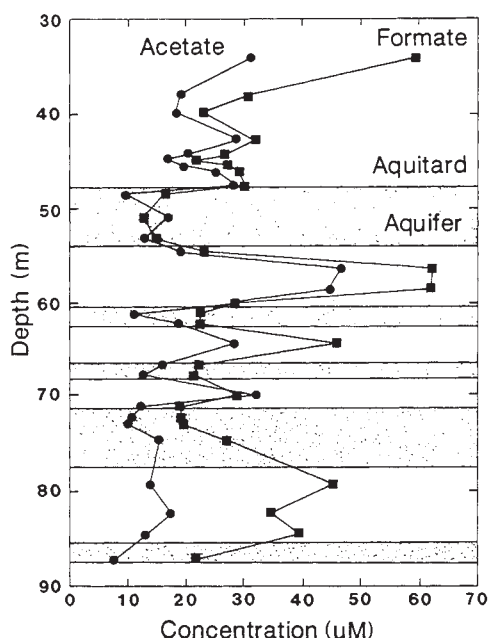


FIG. 1 Concentrations of dissolved formate and acetate in pore water of aquitard and aquifer sediments. Pore-water collection procedures are described elsewhere²⁴. Organic acid concentrations were determined by ion chromatography within 12 hours of sample collection.

(ref. 17), R_{CO_2} is the CO_2 production rate in aquifers from formate and acetate oxidation coupled to sulphate reduction ($mmol\ l^{-1}\ yr^{-1}$), T is the average aquifer thickness (3.0 m) in Fig. 1, and ϕ_s is the effective porosity of aquifer (sand) sediments (0.25). The rate of microbial CO_2 production in aquifers is estimated from equation (2) to be $3.6 \times 10^{-5}\ mmol\ l^{-1}\ yr^{-1}$, which compares favourably with an independently calculated rate of $3.7 \times 10^{-5}\ mmol\ l^{-1}\ yr^{-1}$ (ref. 11), based on the mass balance of dissolved inorganic constituents along an aquifer flow path through the Lake City site. Clearly, the diffusive flux of organic acids from aquitards at this site is sufficient to account for observed respiratory activity in the aquifers. But how are the high organic acid concentrations in the aquitards maintained? The diffusivities of formate and acetate are similar to that of chloride¹⁹. Thus, based on the amount of dissolved chloride in the aquitard sediments ($\sim 290\ \mu M$), and assuming the molar ratio of formate and acetate to chloride in these marine sediments at the time of the last marine incursion was similar to the ratio in modern marine sediments²¹, the organic acid

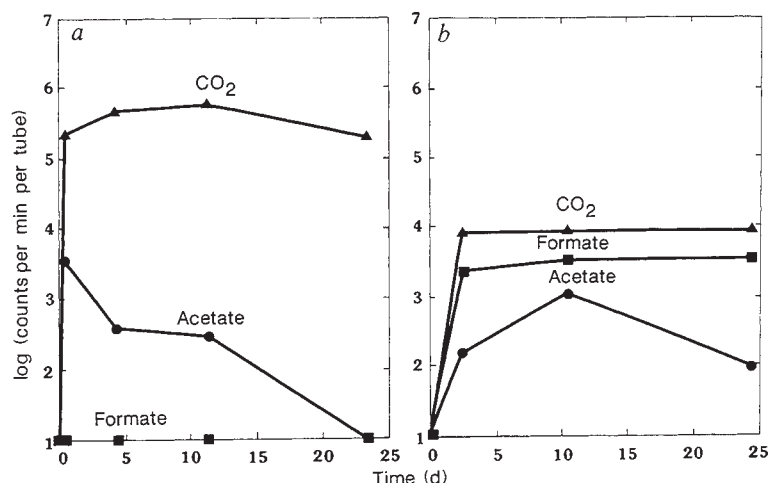
concentration in the aquitards should be only $\sim 0.3\ \mu M$. The fact that the concentrations are at least 200 times higher than this demonstrates that organic acids are actively produced in the aquitards.

We investigated the origin and fate of organic acids in aquifers and aquitards in a series of laboratory incubations of uncontaminated, cored sediment from the Lake City site (Fig. 2). At the beginning of the incubation, acetate accumulated in the aquifer pore water as a result of microbial degradation of glucose (Fig. 2a), but by the end of the experiment it was completely mineralized to CO_2 . In the same experiment, formate production was not observed (Fig. 2a). When unlabelled acetate and formate were added to slow the turnover of the labelled compounds, labelled formate was detected, although in lesser amounts than acetate. The absence of formate in the initial experiment (Fig. 2a) was therefore due to its rapid mineralization to CO_2 rather than to a lack of production. Our results show that glucose degradation in aquifer sediments proceeded according to $glucose \rightarrow formate$ and $acetate \rightarrow CO_2$, with no accumulation of organic acids in the pore water. These data are consistent with the observed low concentrations of organic acids in the aquifers, and demonstrate that microbial respiration maintains aquifer sediments as a sink for organic acids.

In contrast to the incubations of aquifer sediment, both labelled formate and acetate were detected in incubations of aquitard sediments (Fig. 2b). In addition, the relative abundances of formate and acetate were the same as observed in the pore fluids of aquitard sediments (Fig. 1). Labelled formate accumulated rapidly in aquitard pore water at the beginning of the incubation and continued to accumulate, although at a slower rate, throughout the incubation (Fig. 2b). Labelled acetate also accumulated rapidly at the beginning of the experiment and then more slowly. The final data point on the acetate curve, however, makes interpretation of the acetate trend equivocal. The decrease in acetate at the last time point represents either acetate consumption by respiring microbes or heterogeneity in the system. Based on data from the aquifer sediments (Fig. 2a), in which formate was rapidly consumed by respiring microbes, we would expect labelled formate to have also shown a decrease at this time point (Fig. 2b), rather than the observed increase, if respiration was significant. Therefore, it is likely that the labelled acetate, like the labelled formate, was not significantly used by respiring microbes in the aquitard sediment.

This conclusion is further supported by time-series measurements of H_2 , another product of microbial fermentation²², in pore water of incubated aquifer and aquitard sediments (Fig. 3). Concentrations of H_2 in aquifer pore water increased rapidly at the start of the incubation, but then decreased to below detection limits (0.1 nM) by the end of the incubation. Because

FIG. 2 Production of ^{14}C endproducts from radiolabelled glucose in a, aquifer and b, aquitard sediments. Each value is the average of measurements made on duplicate tubes. An anaerobic aqueous solution (2 ml) of radiolabelled glucose was injected into each tube containing $\sim 2.0\ g$ wet sediment to provide $0.5\ \mu Ci$ (ref. 25). Incubations were in the dark at $25^\circ C$. At specified times, sediment and solution in tubes were slurried, centrifuged and supernatant collected. Radiolabelled glucose, formate and acetate in supernatant were separated by ion chromatography. The eluent was collected at predetermined intervals, and ^{14}C measured by liquid scintillation counting. Radiolabelled- CO_2 experiments were performed in separate, duplicate tubes. At specified times, tubes were acidified with $6\ M\ H_3PO_4$, evolved $^{14}CO_2$ collected in KOH basetraps, and ^{14}C measured. Recovery efficiencies of $^{14}CO_2$ in sediments were determined using $H^{14}CO_3$. Reported values were corrected accordingly. All measurements at each time were corrected for ^{14}C activity detected in duplicate, heat-sterilized controls.



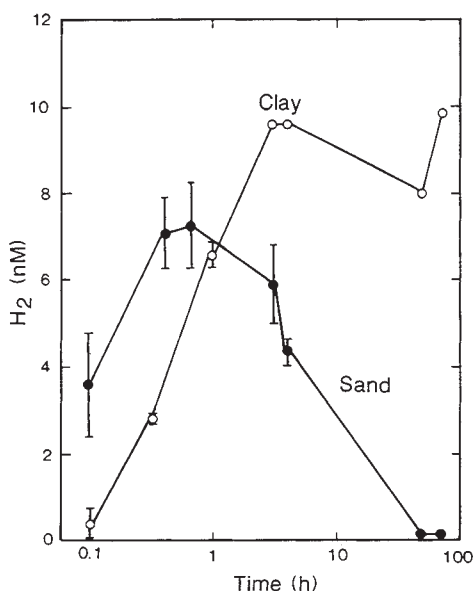


FIG. 3 Concentrations of dissolved hydrogen (H_2) in pore water of aquifer (sand) and aquitard (clay) sediments. Sediment was aseptically transferred to serum vials preflushed with N_2 immediately after core recovery, and hydrogen (H_2) concentrations were determined at specified times using a gas chromatograph equipped with a reduction gas detector²². Each data point represents measurements on duplicate vials.

these sediments are sulphate-reducing^{16,17}, we would expect the steady-state H_2 concentration in them to have stabilized at ~ 2 nM (ref. 22), rather than at concentrations < 0.1 nM. This suggests that the fermentative capacity of these isolated aquifer sediments is not sufficient to maintain microbial respiration at sulphate-reducing levels. In the aquitard sediments, however, H_2 production was initially rapid and continued, at a slower pace, throughout the experiment, achieving concentrations well in excess of 2 nM. This indicates that there was no consumption of H_2 by sulphate-reducing microorganisms and that the fermentative capacity of the aquitard sediments is sufficient to maintain H_2 concentrations in excess of sulphate-reducing levels. Although H_2 concentrations in the aquitard sediments approach levels associated with methanogenesis²², high dissolved sulphate concentrations in the pore water^{16,17} and lack of methane production during the experiment indicate that methanogenic activity in the aquitards was not significant either.

Finally, it is unlikely that the persistence of labelled organic acids in aquitard sediments, and not in aquifer sediments, is due to depressed turnover of the label by the high *in situ* concentrations of formate and acetate. The *in situ* concentrations of formate and acetate in the aquitard sediment were only 4 times greater than in the aquifer sediment, yet the ratio of labelled formate and acetate to CO_2 in the aquitard sediment at the end of the experiment was over 1,000 times greater than that in the aquifer sediment (Fig. 2).

The diffusion of organic acids from aquitards to aquifers links the fermentation of sedimentary organic matter in aquitards to respiratory CO_2 production in aquifers and provides a driving mechanism for microbially mediated changes in the water chemistry in aquifers. In fact, the close agreement between the diffusion-derived CO_2 production rate and the rate derived from mass balance strongly suggests that respiratory activity in the aquifers is limited by the diffusion of organic substrates from aquitards. This, in turn, may explain why CO_2 production rates in different hydrological and geological environments are observed to be so similar¹⁷. In addition to these geochemical considerations, dissolution of aquifer sediments by microbial

CO_2 and organic acids from this process may explain the widely observed phenomenon of increased secondary porosity development at sand/shale contacts²³. □

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Changes in colour appearance following post-receptoral adaptation

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CURRENT models of colour vision assume that colour is represented by activity in three independent post-receptoral channels: two encoding chromatic information and one encoding luminance¹. An important feature of these models is that variations in certain directions in colour space modulate the response of only one of the channels. We have tested whether such models can predict how colour appearance is altered by adaptation-induced changes in post-receptoral sensitivity. In contrast to the changes predicted by three independent channels, colour appearance is always distorted away from the direction in colour space to which the observer has adapted. This suggests that at the level at which the adaptation effects occur, there is no colour direction that invariably isolates only a single post-receptoral channel.

We used an adaptation procedure designed to desensitize post-receptoral channels without significantly changing the sensitivity of receptors^{2,3}. Subjects viewed a uniform field that was slowly modulated in chromaticity and/or luminance along a particular direction in colour space. The modulation does not change the average luminance or chromaticity of the field over time and therefore produces little of the average changes in receptor quantal absorptions that are a major factor in classical chromatic adaptation⁴, yet exposure to it raises thresholds for detecting subsequently presented chromatic or luminance modulations. Krauskopf *et al.*³ found that these sensitivity losses are highly selective if the adaptation is confined to one of three 'cardinal' directions in colour space: an achromatic axis (along which luminance varies while chromaticity is constant), and two equiluminant chromatic axes that selectively modulate either signals from short-wavelength cones (S), or opposing signals

from long- and medium-wavelength cones (L-M). The two chromatic axes stimulate two subsystems of colour vision that evolved at different times⁵: an ancient dichromatic subsystem originally based on a comparison of signals from S cones and from a single ancestral long-wave pigment, and a modern subsystem that evolved to compare the signals of L and M cones after the recent differentiation of the genes encoding their pigments⁶. In many perceptual tasks these postulated subsystems behave independently^{7-10,24}, yet conditions revealing interactions between them have also been found¹¹⁻¹⁴.

For our experiments, stimulus variations that corresponded to the luminance, S, and L-M axes were empirically defined on a colour monitor (as in ref. 15) and were scaled so that a unit distance along each axis equalled the threshold for detecting a change away from the white background. For each of the planes defined by these axes we then selected up to 16 test stimuli, which were spaced on a circle at 22.5° intervals and were each 17 threshold units from the background (Fig. 1). The adapting stimuli were 1 Hz sinusoidal modulations along one of the corresponding eight directions and varied over a range of ± 48 times threshold. The adapting and test stimuli were alternately presented in the same 2° field, but were separated in time by blank periods (1 s before and 0.5 s after each test). The field was centred 1.2° from fixation, and was delineated by narrow black borders from the background, which had the same average colour. Observers initially adapted for 3 min to the adapting modulation. One of the test stimuli was then presented for 0.5 s in the same field, and its perceived colour was matched by

adjusting the colour of a matching stimulus presented simultaneously in a second 2° field, placed symmetrically to the other side of fixation. The adjustments were made during 6 s of re-adaptation before each presentation of the test stimulus.

Figure 2 shows examples for one observer (M.W.) of the colour changes we found following adaptation to four different directions within the equiluminant plane. In Fig. 2a the adapting variation was along either the S or the L-M direction, whereas in Fig. 2b the adaptation varied along intermediate directions chosen to stimulate equally the S and L-M axes. In each case the effect of the adaptation is highly selective: the largest changes were for test stimuli lying along the adapting axis while the least change was for stimuli about 90° away. Very similar effects were found for four further adapting directions, each 22.5° from the S or L-M direction. If the channels adapt independently, then the observed colour changes require at least eight different channels tuned to eight different chromatic directions. Residual selectivity of the adaptation effect for chromatic directions intermediate to the S and L-M axes is also evident from the changes that adaptation produces in chromatic detection thresholds¹¹, yet it is surprisingly salient in the present measures of supra-threshold colour appearance. Ellipses fitted to the matches suggested that for two observers the selectivity for intermediate adapting directions was either comparable to (A.S.) or only slightly less than (M.W.) the selectivity for the S or L-M directions (Fig. 2c). For two further observers (J.M., A.E.), however, the colour changes were clearly more selective for the S and L-M directions, confirming the special status of these directions.

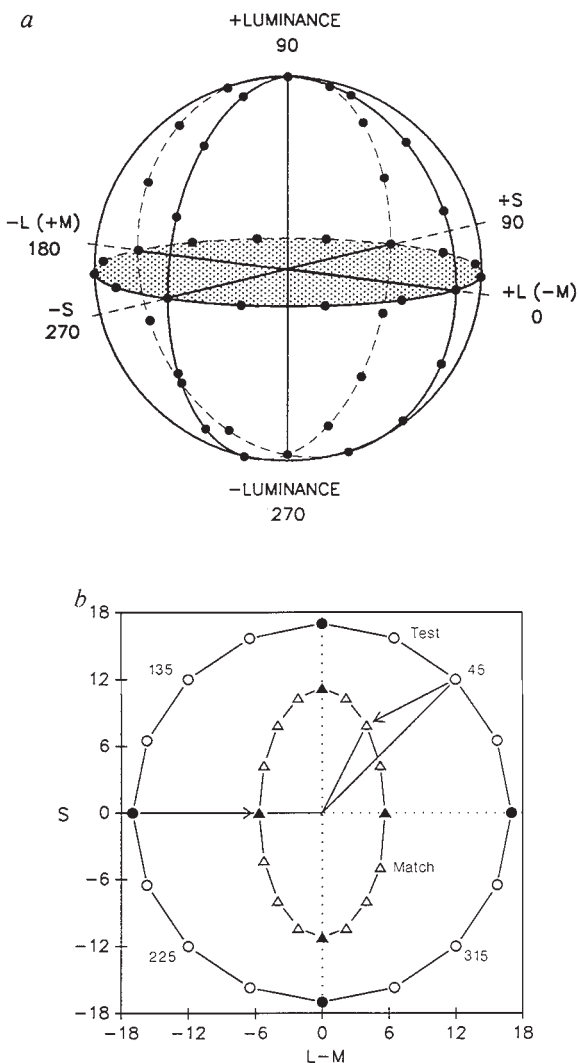


FIG. 1 a, Coordinates of test stimuli in a colour space defined by S, L-M, and luminance axes^{21,23}. The S and L-M axes define an equiluminant plane. Within it distances from the white origin correspond very roughly to saturation while direction corresponds to hue. The achromatic axis corresponds roughly to lightness. b, Predicted colour changes for two independent channels tuned to the S or L-M axis. Adaptation might change colour appearance by reducing the sensitivity of one or both mechanisms. The least or greatest change should be along the S or L-M axis. Sensitivity changes could also distort perceived direction by changing the relative responses to stimuli encoded by both channels (open symbols), but should not affect the perceived direction of stimuli encoded by only one channel (closed symbols).

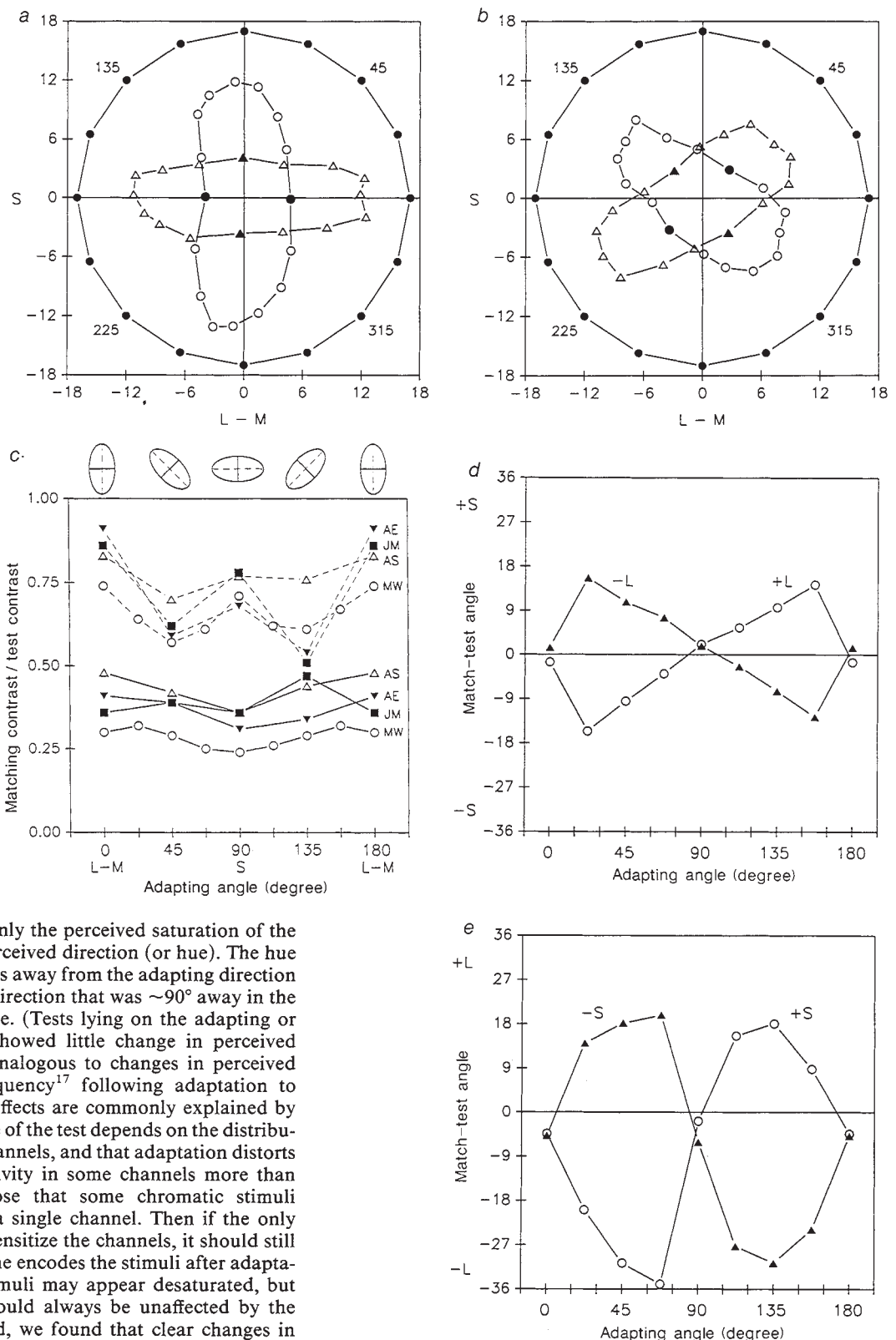


FIG. 2 Coordinates of equiluminant test stimuli (●) and of matches made to them following adaptation to different chromatic axes. In *a*, adaptation was to the L-M (○) or S (△) axis. In *b*, adaptation was to an intermediate axis at 45–225° (○) or 135–315° (△). Matches to tests that were on the adapting axes are indicated by filled symbols. *c*, The match coordinates for each adapting direction fall approximately on an ellipse, which was fitted to estimate the change in perceived contrast along the adapting axis (—) and orthogonal axis (---) for each of the four observers tested (the two authors and two naive subjects). *d* and *e*, Change in perceived direction of the two test stimuli lying on the L-M (*d*) or S axis (*e*), as a function of the direction of the adaptation.

Adaptation changed not only the perceived saturation of the test stimuli, but also their perceived direction (or hue). The hue changes were always rotations away from the adapting direction and towards an orthogonal direction that was $\sim 90^\circ$ away in the normalized S and L-M plane. (Tests lying on the adapting or orthogonal axes generally showed little change in perceived angle.) These changes are analogous to changes in perceived orientation¹⁶ or spatial frequency¹⁷ following adaptation to spatial patterns. Such after-effects are commonly explained by assuming that the appearance of the test depends on the distribution of activity in multiple channels, and that adaptation distorts appearance by reducing activity in some channels more than others^{18,19}. However, suppose that some chromatic stimuli change the activity in only a single channel. Then if the only effect of adaptation is to desensitize the channels, it should still be the same channel that alone encodes the stimuli after adaptation. Consequently, such stimuli may appear desaturated, but their perceived direction should always be unaffected by the adaptation (Fig. 1*b*). Instead, we found that clear changes in perceived angle could be induced in all of the test stimuli we examined. Examples of these changes are shown in Fig. 2*d* and *e* for the two tests lying along either the L-M or the S axes. These are the tests most likely to isolate a single adaptable channel, yet adaptation to intermediate chromatic axes rotated the perceived angle of these tests off their axes. Similar distortions were found for all the test stimuli, including stimuli chosen to lie along the unique hue loci, and occurred for all four observers. This suggests that there are no chromatic directions encoded by only a single adaptable chromatic channel.

Surprisingly, adaptation also produced changes in colour appearance that were selective for stimuli varying in both luminance and chromaticity, despite previous findings that the adaptation influences detection thresholds for these dimensions independently³. For example, Fig. 3 shows for observer M.W. the effects of adapting to modulations along either a pure luminance or a pure chromatic (L-M) axis (Fig. 3*a*), or along two directions for which luminance and chromaticity covaried (Fig. 3*b*). Again, the adaptation produced the largest sensitivity losses

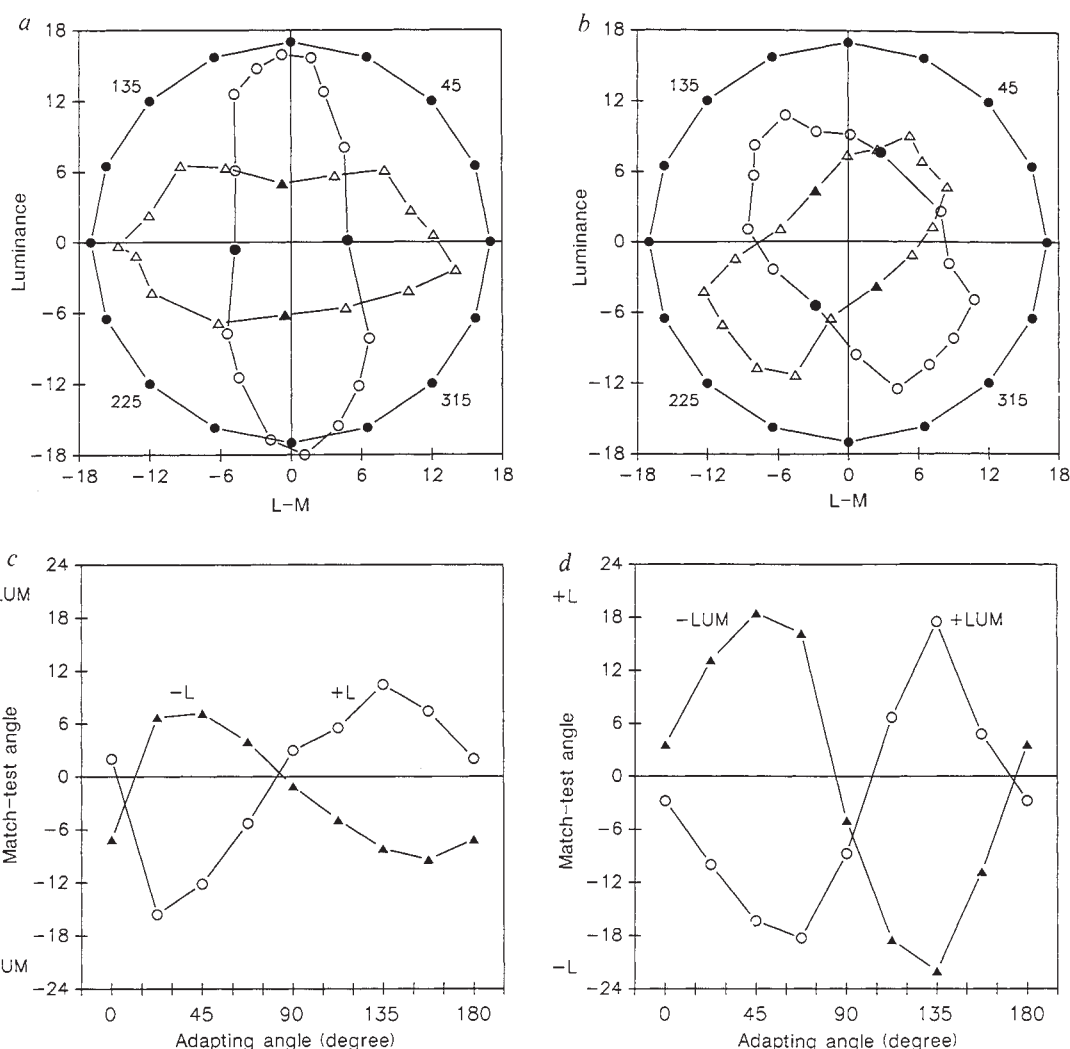


FIG. 3 *a*, Matches made to test stimuli in the L-M/luminance plane following adaptation to the L-M (○) or luminance (△) axis. *b*, Matches made to the same tests following adaptation to combined luminance and chromatic variations along axes at 45–225° (○) or 135–315° (△). *c* and *d*, Changes in the perceived direction of the two pure chromatic tests (*c*) or the two pure luminance tests (*d*) for different adapting directions.

for test stimuli that were near the adapting axis (a result confirmed for a second observer, A.S.). This selectivity was also evident when the chromatic component modulated activity only in the S cones, even though the S cones contribute very little to measures of luminance²⁰, and are often assumed to have access only to purely chromatic pathways. Adaptation to combined luminance and chromatic variations also produced changes in the perceived direction of luminance and/or chromatic stimuli. For example, adaptation could alter the relative lightness of equiluminant chromatic tests (Fig. 3c) and the relative hues of tests that had the same chromaticity but differing luminances

(Fig. 3d), suggesting that these stimuli were not encoded only by purely chromatic-sensitive or luminance-sensitive channels.

These selective adaptation effects probably have a cortical locus, as physiological studies suggest that lateral geniculate neurons are little adapted by the type of stimuli we used²¹. Our results could be explained by the existence of multiple, adaptable channels, each tuned to a different direction in colour space¹¹. Alternatively, however, central chromatic channels might be coupled through adaptation-dependent links that alter their tuning functions²². □

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Allele- and haploid-specific product generated by alternative splicing from a mouse *t* complex responder locus candidate

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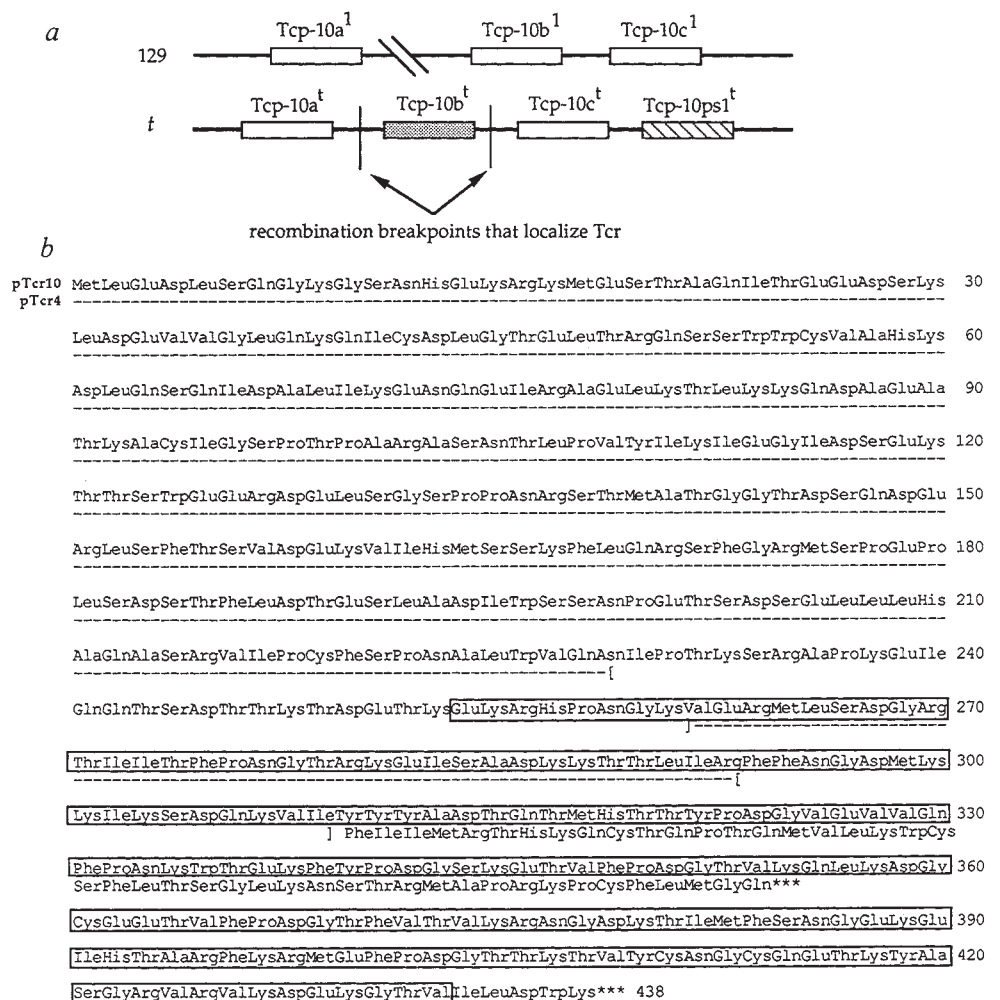
MOUSE *t* haplotypes represent a variant form of chromosome 17 that has evolved the ability to propagate through natural populations by the phenomenon of 'transmission ratio distortion' (TRD), in which heterozygous $+/t$ males transmit their *t*-carrying chromosome to 95% or more of their offspring^{1,2}. Although multiple *t*-associated loci have a role in expression of this phenotype, only one—the *t* complex responder (*Tcr*) locus—is responsible for determining which of the two homologues of chromosome 17 will be transmitted at a high ratio³. A candidate gene (*Tcp-10b*) for *Tcr*

that is expressed in both meiotic and post-meiotic male germ cells has been cloned⁴. But for this candidate gene to function as the haploid effector of TRD, the *t*-allele of this gene (*Tcp-10b^t*) must express a unique product in a haploid-specific manner. Here we show that a change in the splicing pattern of *Tcp-10b^t* transcripts occurs during sperm differentiation. This change results in a unique allele-specific and haploid-specific transcript which could encode a variant polypeptide that would fulfil the conditions required of the *Tcr* effector of TRD.

During our studies of the *Tcp-10* gene family, we recovered a complementary DNA clone (pTcr4) clearly derived from the *t*-allele of the candidate *Tcr* gene, *Tcp-10b^t*, as determined by sequence analysis⁴ (Fig. 1), but missing one whole exon and a portion of a second exon as a result of the use of a cryptic splice donor site (CAG/GTTTTT, Fig. 2b). We used polymerase chain reaction (PCR) to investigate whether this variant *Tcp-10b^t* messenger RNA is correlated with the phenotype associated with the *Tcr^t* allele. Three bands are observed when gel-fractionated *Tcp-10* PCR products obtained from total testes RNA are probed with a *Tcp-10b^t* allele-specific oligonucleotide (Fig. 2a, lanes 1 and 4). The size of the largest PCR product (no. 1) corresponds to that expected from the full-length *Tcp-10* sequence seen with many cDNA clones (1,298 nucleotides⁴).

FIG. 1 a, Organization of the *Tcp-10* gene family on wild-type (129/SvJ) and *t* haplotype forms of chromosome 17. The 129/SvJ alleles at each locus are designated by a '1', to distinguish them from other wild-type (+) alleles. The most proximal member of the wild-type gene family (*Tcp-10a*) has become separated from the others as a result of a paracentric inversion with a distal breakpoint within the gene family⁶. High-resolution mapping of the *Tcr* locus demonstrates its localization to the region containing the *Tcp-10b* gene only^{7,8}. *Tcp-10ps1^t* is a non-expressed pseudogene. **b**, Sequence comparison of the open reading frames encoded by two cDNA clones—pTcr10 and pTcr4—derived from alternatively spliced transcripts of the *Tcp-10b^t* allele. The pTcr10 sequence has been previously published⁴. The pTcr4 clone shares 5 *Tcp-10b^t* allele-specific base changes with pTcr10 and an additional four changes that distinguish it from the *Tcp-10a^t* or *Tcp-10c^t* genes (positions 123, 161, 264, 359, 651, 679, 801, 804, and 943). Nucleotide numbers here and below are given relative to the first ATG of the conserved open reading frame (previously defined as 421 by Schimenti *et al.*⁴). The repeating motif region is boxed. Amino-acid identities are indicated by a —, and deleted regions are delineated by square brackets.

METHODS. pTcr10 was derived from a conventional cDNA library obtained from heterozygous congenic 129/+/t^{w5} testicular RNA as described previously⁴. To derive pTcr4, total testicular RNA obtained from a t^{w5}/t^{w18} mouse was isolated by the guanidium thiocyanate method followed by pelleting through CsCl⁹. Haplotype t^{w18} is a partial *t* haplotype derived from t^{w5}; t^{w5}/t^{w18} is effectively homozygous for the *Tcp-10^t* gene family. First strand cDNA from 10 µg total RNA was produced with avian myeloblastosis virus reverse transcriptase from an internal primer, and was amplified using *Taq* polymerase



(Cetus) according to the manufacturer's recommendations. The amplified product was purified, cut at internal *Eco*RI and *Xho*I restriction sites, and cloned into plasmid pBluescript (Stratagene). Plasmids were sequenced by the dideoxynucleotide method (Sequenase, USB).

The size of the second largest PCR product (no. 2) corresponds to that expected from the transcript that gave rise to the variant pTcr4 clone (1,153 nucleotides).

To verify that the PCR product did indeed have the same structure as pTcr4, the blot was rehybridized sequentially with two oligonucleotides that define the two variant regions of this clone. The first oligonucleotide (VII $\rightarrow$ IX) is complementary to sequences on both sides of the variant exon VII-exon IX junction found in pTcr4. Positive hybridization to PCR product no. 2 with this splice-specific oligonucleotide (Fig. 2a, lane 2) demonstrates that exon VIII has been spliced out from the corresponding transcript. The second region-specific oligonucleotide (IXB) is complementary to a 21-nucleotide sequence present in the 3' portion of exon IX which is also spliced out from pTcr4. The IXB oligonucleotide is not allele-specific and cross-hybridizes to products from other *Tcp-10* genes. This oligonucleotide fails, however, to hybridize with PCR product no. 2 (Fig. 2a, lane 3). Together, the size determination and PCR hybridization data indicate that PCR product no. 2 has

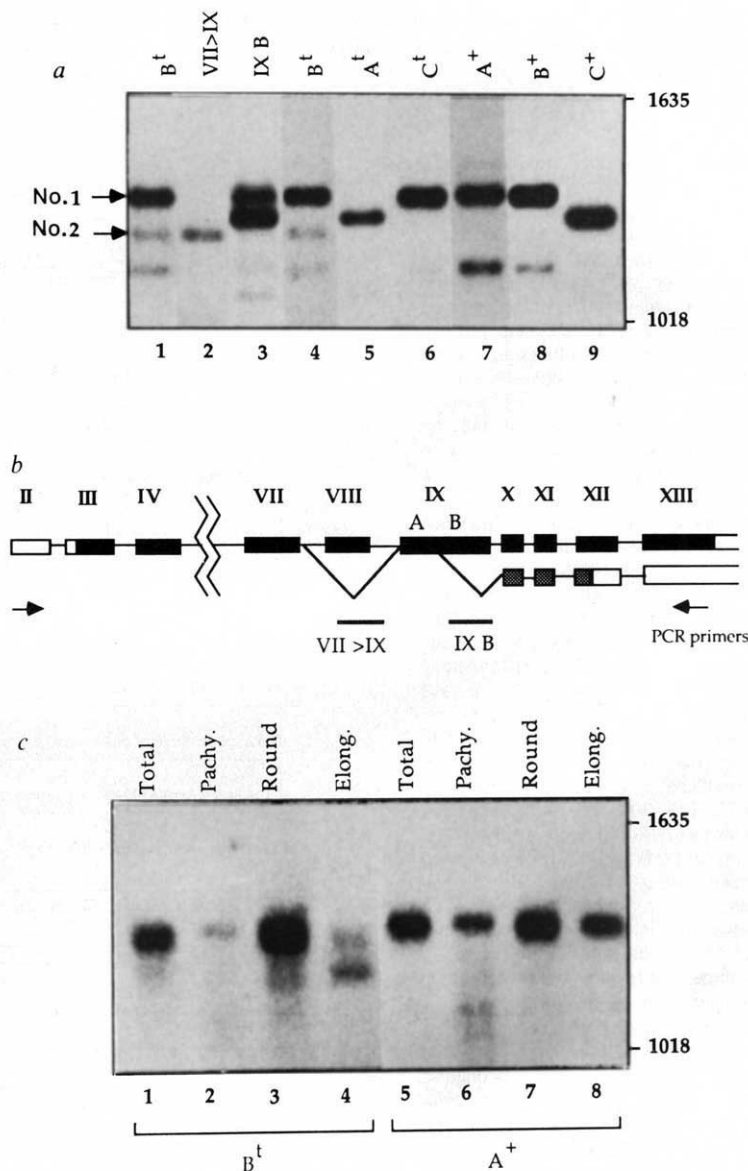
the same structure as the variant *Tcp-10b*^t clone, pTcr4.

To determine whether this alternatively spliced product is associated solely with transcripts derived from the *Tcp-10b*^t allele, total *Tcp-10* PCR products from a +/t heterozygous mouse were sequentially hybridized with allele-specific oligonucleotides corresponding to each of the three functional members of the gene family present in both wild-type and t chromosomes. The variant transcript corresponding to the pTcr4 clone is produced uniquely by the *Tcp-10b*^t allele (Fig. 2a).

To investigate whether the variant *Tcp-10b*^t transcript is expressed in a stage-specific manner, spermatogenic cells were fractionated into pre-meiotic and post-meiotic fractions by unit gravity sedimentation before RNA purification and analysis. With a *Tcp-10b*^t allele-specific oligonucleotide, one can demonstrate the expression of the variant transcript only in the haploid cell fractions (Fig. 2c). This observation indicates that changes in the splicing machinery must occur during sperm differentiation. As the wild-type *Tcp-10* genes do not undergo a temporal change in splicing patterns (Fig. 2c), it seems that *Tcp-10b*^t is

FIG. 2 PCR analysis of *Tcp-10* transcription. a, Analysis of PCR-amplified *Tcp-10* RNA from whole testes with oligonucleotide probes. All oligonucleotides are described in Table 1. Lanes 1-3 represent the amplified products from t^{w5}/t^{w28} testicular RNA sequentially hybridized with oligonucleotides specific for (1) *Tcp-10b*^t, (2) the variant VII $\rightarrow$ IX splice junction, and (3) the 3' end of exon IX (defined below). Lanes 4-9 represent the amplified products of +/t^{w5} testicular RNA sequentially hybridized with each of the allele-specific oligonucleotides as indicated above each lane. The smallest echo band present in each lane is not observed in PCR amplifications from oligo(-dT) primed cDNA (see Fig. 2c), and seems to be an artefact generated during cDNA priming. The major product of the *Tcp-10a*^t allele (lane 5), which is smaller than those from the other *Tcp-10* alleles, and is similar but not identical in size to the *Tcp-10b*^t variant product, is associated with an unrelated deletion in the 5' end of the transcript (nucleotides 116 $\rightarrow$ 175). b, Schematic representation of the exon-intron structure corresponding to the two alternatively spliced forms of the primary *Tcp-10b*^t transcript. (All intron-exon borders have been determined by D. Bullard and J. Schimenti, personal communication). Coding regions associated with each transcript type are shaded. Locations of the two PCR primers used for amplification are indicated by arrows (see Table 1). These primers correspond to nucleotide sequences shared by all members of the *Tcp-10* gene family on both wild-type and t haplotype chromosomes. The VII $\rightarrow$ IX oligonucleotide is complementary to an 18-nucleotide sequence extending across the variant splice junction between exons VII and IX. Only transcripts that have spliced out exon VIII will hybridize to this oligonucleotide. Oligonucleotide IXB derives from the 3' portion of exon IX which can be eliminated by alternative splicing. c, PCR analysis of *Tcp-10* splicing patterns during germ cell differentiation. Enriched germ cell populations were obtained from +/t^{w5} testes. Germ cell stages are indicated above each lane (Pachy, pachytene spermatocytes; Round, round spermatids; Elong., elongating spermatids). PCR products were hybridized sequentially with the *Tcp-10b*^t and *Tcp-10a*^t-specific oligonucleotides.

METHODS. First strand cDNA was produced from total testicular RNA and amplified as described above. The PCR products were electrophoresed through 1.2% agarose (Seakem, FMC Bioproducts) and transferred to Genescreen (Dupont) by standard methodologies. The blots were probed with oligonucleotides labelled at their 5' ends (see Table 1). Isolated testicular germ cells were fractionated by unit gravity sedimentation over a 2-4% linear BSA gradient¹⁰ using a Celsp apparatus (Dupont). Individual fractions were assessed microscopically, and pooled. The purity of the pools was assessed after staining with Mayer's haematoxylin (Sigma). The pachytene spermatocyte fraction was 72% pure, and was primarily contaminated with multinucleate round spermatids. The round and elongating spermatid fractions were 75% and 56% pure respectively, and were primarily



contaminated with each other. Pooled pachytene spermatocytes, round spermatids and elongating spermatids were lysed by boiling in H₂O in the presence of diethylpyrocarbonate, and the clarified lysate was reverse-transcribed with oligo(dT) as a primer, and PCR-amplified as described above.

TABLE 1 Oligonucleotides and primers

Target	Nucleotide no.*	Corresponding cDNA sequence†
<i>Tcp-10a</i> [‡]	107 → 115/176 → 187‡	GGCTGCAGA/ACAAAACCTCC
<i>Tcp-10b</i> [‡]	349 → 368	GATTCGAGAAGACAACCTC
<i>Tcp-10c</i> [‡]	416 → 438	CAATGGCCACCAGGAACACAG
<i>Tcp-10a</i> ⁺	512 → 530	GAAGCTTGGCAGAAATGTC
<i>Tcp-10b</i> ⁺	444 → 464	GATGAAATGTTGTCTTTAC
<i>Tcp-10c</i> ⁺	—44§	CAAGAATGCACGAGCCTTCCC
VII → IX	675 → 684/784 → 792	GTGGGTGCAG/GTGGAGCGG
Exon IXB	892 → 912	GACATGAAGAAGATCAAGTCC
5' primer	—162 → —141	CCCTCTCAAGGCCTGTTCACCTC
3' primer	1,203 → 1,221	CCCAGATGGCACCACCAAG

* Location of each oligonucleotide relative to the first ATG of the conserved open reading frame (previously nucleotide 421 in Schimenti *et al.*⁴).

† Allele-specific base changes are underlined.

‡ Crossing the *Tcp-10a*⁺ allele-specific deletion.

§ 21 nucleotides of an allele-specific 30-base pair insertion at the 5' end of exon III (S.H.P. *et al.*, manuscript in preparation).

using a process that has evolved for other purposes. The implication is that changes in the splicing machinery occur normally to alter other patterns of protein expression during spermatogenesis. Two unanswered questions raised by these results are: (1) what is the nature of the *trans*-acting factors that regulate the *Tcp-10b*⁺ splicing switch; and (2) what are the *cis*-acting elements within the *Tcp-10b*⁺ primary transcript that distinguish it from the products of the other *Tcp-10* genes and allow it to follow this change in splicing pathway?

The putative polypeptide encoded by the variant *Tcp-10b*⁺ transcript has a structure strikingly different from that of the polypeptide produced by the full-length mRNA (Fig. 1b). The removal of exon VIII causes the in-frame deletion of 33 amino acids that seem to form a single, hydrophilic domain. The removal of the 3' end of exon IX changes the reading frame, resulting in the elimination of 145 amino acids from the C terminus of the protein product, and its replacement with an unrelated stretch of 45 amino acids. In previous studies, we found that the C terminus of the full-length polypeptide is composed of 20 repeats of a basic 9-residue motif⁴. We have compared mouse and human homologues of *Tcp-10*, and found that this repeating motif region is the most highly conserved portion of the polypeptide (S. D. Islam, S.H.P., C.L.D. and L.M.S., manuscript in preparation).

These changes in TCP10Bt protein structure are likely to have dramatic effects on protein function. This would be consistent with current views of the action of the *Tcr*⁺ product which is thought to 'protect' *t*-bearing spermatids from the deleterious effects of the *Tcd*⁺ products^{3,5}. It has been proposed that *Tcd*⁺ products interact unfavourably with the *Tcr*⁺ product causing the dysfunction of *t*-spermatids. If the variant TCP10Bt product did indeed function as the *Tcr*⁺ effector, its action could be accounted for within the context of this model by hypothesizing that it is unable to bind *Tcd*⁺ products as a result of its altered structure, although it retains the ability to perform the normal *Tcr*⁺ role in spermatogenesis. □

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Participation of CD4 coreceptor molecules in T-cell repertoire selection

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DURING thymocyte development, progenitor cells bearing both CD4 and CD8 coreceptor molecules mature into functional T lymphocytes that express these proteins in a mutually exclusive way¹. Although T-cell specificity is determined primarily by the structure of the T-cell antigen receptor (TCR) heterodimer², a developmentally regulated process acts to ensure that cells bearing class II-restricted TCRs are CD4⁺ and those bearing class I-restricted TCRs express only CD8 (ref. 3). To investigate this maturation process, we have engineered transgenic mice in which CD4 is expressed in all thymocyte subsets and in all peripheral T cells. Peripheral CD4⁺ T lymphocytes from these mice react with both class I and class II alloantigens. Moreover, expression of the CD4 transgene disrupts the positive selection of doubly transgenic thymocytes bearing a class I-restricted TCR specific for the male (H-Y) antigen. Hence the CD4 coreceptor participates directly in T-cell repertoire selection.

To augment expression of CD4 in thymocytes, a murine CD4 minigene was fused to the proximal promoter of the murine *lck* gene (Fig. 1). The proximal *lck* promoter is expressed at high levels in the thymus^{4–6} and a 3.2-kilobase fragment of this promoter directs thymocyte-specific expression of heterologous genes in transgenic mice^{7–9}. Two independent lines of *lck*-CD4 transgenic mice were established that express high levels (15–30 times normal) of CD4 on thymocytes. In general, this alteration in CD4 expression does not grossly affect thymocyte development (see below).

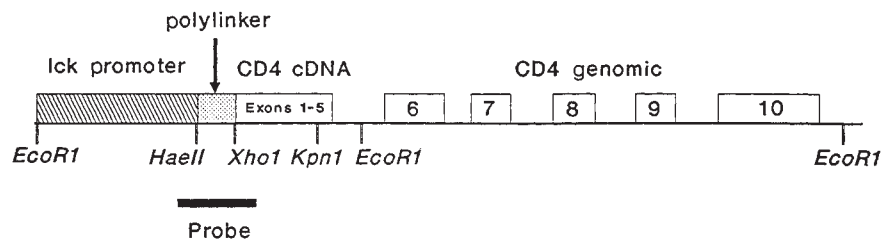
To determine whether the repertoire of mature CD8⁺ T cells can be altered by expression of the CD4 transgene, CD4 transgenic mice (line 727) were mated with mice expressing a transgenic $\alpha\beta$ TCR specific for the H-Y antigen presented by H-2D^b class I molecules¹⁰. Thymocytes and lymph node cells from female $\alpha\beta$ and doubly transgenic $\alpha\beta$ /727 mice were analysed by three-colour staining with monoclonal antibodies directed against CD4, CD8, and either the α (T3.70; refs 11, 12) or the β (F23.1; ref. 13) TCR transgene product. In $\alpha\beta$ transgenic mice, the β transgene product is expressed by all T cells, whereas the α transgene product is expressed preferentially on CD4⁺ T lymphocytes. This preferential expression results from intrathymic positive selection by H-2D^b class I molecules^{11,14}.

In accord with previous reports (refs 11, 12), mature CD4⁺ T cells were overproduced in the thymuses of $\alpha\beta$ transgenic mice (47% of thymocytes as compared with 3% for normal mice; see Fig. 2). Positive selection of CD8⁺ T cells was substantially reduced in $\alpha\beta$ /727 thymuses—only 11% of thymocytes were mature CD8⁺ cells. Note that during the transition from immature thymocytes to mature T cells, the expression of the *lck*-CD4 transgene is reduced by ~15-fold (unpublished data). This is consistent with the finding that the proximal *lck* promoter

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FIG. 1 Structure of the *lck*-CD4 transgene. The proximal *lck* promoter (−3,200 to +37 base pairs (bp) with respect to the transcription start site) was fused to a murine CD4 minigene. The minigene consists of a 500 bp *Xho1/Kpn1* fragment from the CD4 complementary DNA²⁶ containing exons 1–5, fused to a 10.3 kilobase pair (kb) *Kpn1/EcoRI* genomic fragment containing exons 6–10. A 65-bp oligomer probe spanning the junction between *lck* and CD4 was used to probe mouse tail genomic DNA to identify animals harbouring the transgene.

Transgenic animals were generated by microinjection of the *lck*-CD4 insert DNA into C57BL/6J×DBA/2 F₂ fertilized eggs as described⁷. Six founder animals were obtained, of which four expressed the transgene. Two lines



of these animals were established by backcrossing founder animals (727 and 813) with C57BL/6J partners.

is most active in immature thymocytes and functions poorly in mature T cells^{4–9}. As a result, mature CD8⁺ T cells from $\alpha\beta$ /727 mice expressed slightly less CD4 than normal CD4⁺8⁺ T cells and had a CD4⁺4⁺8⁺ phenotype (where E represents endogenous and T, transgenic). Also, only 23% of CD4⁺8⁺ thymocytes from $\alpha\beta$ /727 mice expressed the transgenic α chain, compared with 96% of thymocytes from $\alpha\beta$ mice (Fig. 2). The remaining cells in each case expressed $\alpha\beta$ TCRs. Similarly, 59% of CD4⁺8⁺ lymph node T cells from $\alpha\beta$ mice expressed the transgenic heterodimer, compared with only 9% of CD4⁺8⁺ T cells from $\alpha\beta$ /727 mice. Hence positive selection of CD8⁺ thymocytes is dramatically attenuated by overexpression of the CD4 coreceptor. A total of four $\alpha\beta$ and six $\alpha\beta$ /727 female mice were analysed in this way, yielding in each case results similar to those shown in Fig. 2. Interestingly, preferential expansion of mature CD8⁺ T cells expressing endogenous TCR α chains was observed in the lymph nodes of both $\alpha\beta$ and $\alpha\beta$ /727 mice, presumably as a result of priming by environmental antigens.

CD4⁺8⁺ and CD4⁺8⁺ T cells are positively selected by thymic class II and class I MHC molecules respectively^{11,14–17} to yield T cells in which TCR restriction and coreceptor expression are complementary. As mature CD8⁺ T cells from 727 and $\alpha\beta$ /727

mice simultaneously express CD4, we determined whether these cells showed class II as well as class I MHC-restricted specificity. Lymph node cells from normal, 727, $\alpha\beta$ and $\alpha\beta$ /727 female mice were stained with anti-CD4 and CD8 monoclonal antibodies and fractionated by two-colour cell sorting. We then determined the proliferative response of the sorted cells to female bm1 (an H-2K^b class I MHC mutant¹⁸), female bm12 (an I-A^b class II MHC mutant¹⁹), and male B6 (H-2^b) antigens. The results of one of four experiments are shown in Fig. 3. CD4⁺8⁺ T cells from normal, 727 or $\alpha\beta$ /727 mice responded to bm12 but not to bm1 stimulators, indicating that these cells were restricted to class II MHC antigens. Similarly, CD4⁺8⁺ T cells from normal and $\alpha\beta$ mice responded to bm1 but not to bm12 stimulators, indicating that they were restricted to class I MHC antigens. CD4⁺8⁺ T cells from $\alpha\beta$ mice also responded dramatically to B6 male stimulator cells, which is consistent with the observation that most of these cells express the male-specific TCR (Fig. 2). Remarkably, CD4⁺8⁺ T cells from 727 and $\alpha\beta$ /727 mice responded to both bm1 and bm12 stimulator cells. Hence, simultaneous expression of CD4 and CD8 coreceptors is sufficient to permit elaboration of peripheral T cells capable of recognizing both class I and class II alloantigens.

It is unclear whether the dual bm1/bm12 reactivity of thymocytes from *lck*-CD4 mice results from natural cross-reactivity in certain TCR structures that is made apparent by the presence of the CD4 coreceptor (and hence of individual cells that simultaneously recognize allogeneic class I and class II determinants), or from the intrathymic selection of CD4⁺8⁺ cells restricted by class II-presenting structures. In the latter case, TCR specificity must be separated from endogenous coreceptor expression, so presumably the complementarity between TCR and coreceptor results from selection acting on pre-existing thymic populations in which all combinations of receptor and coreceptor specificity are available²⁰.

Regardless of the mechanism responsible for the emergence of CD8⁺ thymocytes capable of recognizing a class II alloantigen, we found that overexpression of CD4 dramatically inhibited positive selection of male antigen-specific CD8⁺ thymocytes. This result indicates that intrathymic signalling from the transgenic $\alpha\beta$ molecules could have been compromised to some extent by the excess CD4. Neonatal administration of anti-coreceptor antibodies disrupts positive selection of thymocytes, presumably by preventing interaction of the coreceptor structure with an appropriate MHC ligand^{21,22}. In $\alpha\beta$ /727 mice, diminished positive selection of H-Y-specific thymocytes probably results from competition between CD4 and CD8 coreceptors for the TCR itself²³, or for some other molecule involved in CD4/CD8 signalling. For example, the protein tyrosine kinase p56^{lck}, which interacts with the cytoplasmic tails of both the CD4 and CD8 coreceptors^{24,25}, might be present at limiting concentrations in thymocytes, and so might preferentially associate with the highly represented transgene-encoded CD4 protein. This would predict that $\alpha\beta$ animals expressing a transgene-encoded CD4 molecule without the cytoplasmic tail would not manifest alterations in the positive selection of H-Y-specific cells.

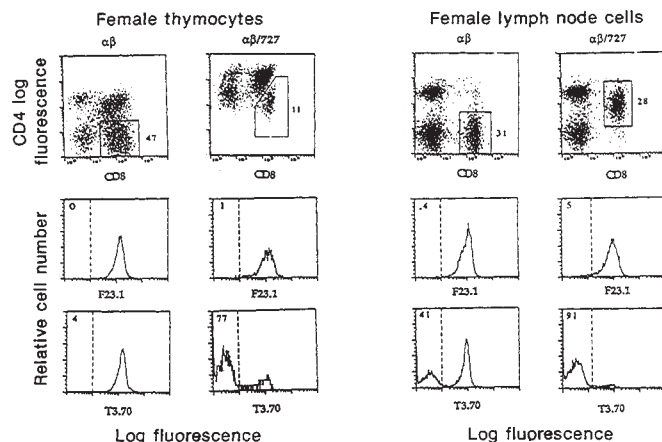


FIG. 2 Perturbation of the TCR repertoire of mature CD8⁺ T cells in CD4 transgenic mice.

METHODS. Thymocytes and lymph node cells from 8-week-old female $\alpha\beta$ and $\alpha\beta$ /727 mice were collected. The yields of thymocytes from $\alpha\beta$ and $\alpha\beta$ /727 mice were $\sim 150 \times 10^6$ and 100×10^6 per thymus, respectively. Three-colour staining of these cells with antibodies directed against CD4, CD8 and the α or β transgene product was performed as follows: cells were incubated first with biotinylated F23.1 antibody (anti- β) or biotinylated T3.70 antibody (anti- α) and next with streptavidin-conjugated duochrome (Becton-Dickinson). Labelled cells were then incubated with a mixture of phycoerythrin-conjugated anti-CD4 and fluorescein-conjugated anti-CD8 antibodies. Triply labelled cells were analysed with a FACScan Flow Cytometer using FACScan research software programs. A total of 15,000 cells per sample were analysed. The plotted histograms correspond to the levels of expression of the β and α transgenes by CD4⁺8⁺ and CD4⁺8⁺ mature T cells from $\alpha\beta$ and $\alpha\beta$ /727 mice, respectively.

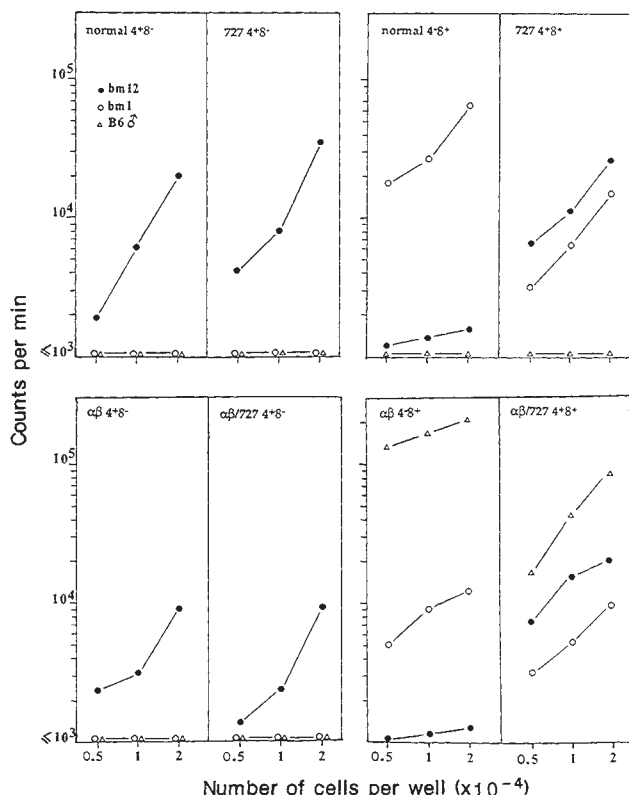


FIG. 3 CD4⁺8⁺ T cells from CD4 transgenic mice respond to both class I and class II alloantigens.

METHODS. Lymph node cells (axillary, brachial, inguinal and mesenteric) were collected from 12-week-old female normal, 727, $\alpha\beta$ and $\alpha\beta/727$ mice and stained with phycoerythrin-conjugated anti-CD4 and fluorescein-conjugated anti-CD8 antibodies. Labelled cells were sorted into CD4⁺8⁺ and CD4⁺8⁺ populations using a FACS IV cell sorter (Becton-Dickinson). Sorted cells were cultured at the indicated numbers in quadruplicate with irradiated (2,000 rads from a ⁶⁰Co source) spleen cells (5×10^5 cells per well) from female bm1 (B6.CH2^{bm1}/ByJ; Jackson Labs), female bm12 (B6.CH2^{bm12}/KhEg; Jackson Labs), male C57BL/6 (B6) or female C57BL/6 mice. Each culture was supplemented with an excess (20 units ml⁻¹) of murine recombinant interleukin-2²⁷. Proliferative responses to the various stimulator cells were assessed at the peak of the proliferative phase (108 h for response to B6 male and bm1 antigens, and 132 h for response to bm12 antigens). One μ Ci of [³H]thymidine was added to each culture 16 h before harvest. The indicated counts for the various test groups are those from which the background interleukin-2 response, as assessed by the proliferative response against B6 female spleen cells (ranging from a few hundred to ~5000 c.p.m.), had been subtracted. The standard errors (not shown) for all groups were within 20% of the mean.

Our data make plain that the peripheral representation of TCR specificities can be dramatically altered by abrogation of the normal mutually exclusive expression of CD4 and CD8. Indeed, we find that the endogenous V β repertoire of CD8⁺ lymph node cells is itself perturbed in *lck*-CD4 mice (A.M.G., K.A.F., D.R.L. and R.M.P., manuscript in preparation). By appropriate manipulation of mice expressing coreceptor transgenes, it should be possible to elucidate the mechanisms that maintain congruence between TCR and coreceptor specificities. □

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Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*

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A WELL recognized feature of the immune response to parasitic helminth infections, including schistosomiasis, is the production of large amounts of specific and nonspecific IgE^{1,2}. Immunological pathways involving IgE can lead to damage to the developing schistosomulum^{3–9} and it has been suggested that responses involving IgE could have evolved as protection against helminth infections^{10,11}. There has been no epidemiological evidence to support this idea and the only significant IgE responses known in man are those involved in the pathogenesis of allergic disease¹². Here we measure serological response during reinfection with *S. haematobium* and demonstrate that IgE antibodies in man can be beneficial. Our results support the hypothesis that the slow build-up of IgE to high levels and the early production of IgG4 antibodies, which may block IgE pathways^{13,14}, are responsible for delaying the development of protective immunity to *S. haematobium*.

The studies were carried out in a small community in The Gambia, West Africa, where the intensity and prevalence of *S. haematobium* infection showed the characteristic pattern of a peak early in the second decade of life and a fall to lower levels later, with the fall in intensity being the more marked¹⁵. The study design is shown in Table 1. Initially we found that reinfection after treatment with praziquantel was frequent and intense in children aged under ten and comparatively rare and of low intensity in subjects older than 15 years (Fig. 1). Water contact was monitored during the study and showed that some adults were as intensely exposed to infection as the children^{16,17}. These observations suggest that a significant degree of protective immunity against *S. haematobium* infection¹⁶ gradually develops in this community, and that the immunological mechanisms responsible for the age-related decline in intensity and prevalence of infection are most developed in subjects aged over 15 years.

We measured serum antibody levels and found relationships between age and levels of IgE and IgG4 schistosome-specific

antibodies (Fig. 2). IgE levels were maximal in the 15+ age group and the age-IgE profile follows that expected for an antibody involved in resistance to infection. By contrast, the levels of IgG4 against worm and egg antigens were highest in the 10-14-year-olds. IgG4 antibody responses to worm and egg antigens were closely correlated ($r=0.82$), although they had a slightly different relationship with age.

The association of IgE and IgG4 antibodies with reinfection was investigated by multiple logistic regression in which terms for age, intensity of exposure to cercariae, and IgE and IgG4 levels were used to predict the probability of reinfection, the latter being considered as a dichotomous variable without reference to infection intensity (Table 2). Terms for age and intensity of exposure to cercariae were included to take into account potential confounding effects of these variables. Reinfection was significantly less likely in those with high levels of IgE antibody against antigens derived from the adult worm and more likely in those with high levels of IgG4 antibodies against either the worm or egg; these effects were sizeable. Reinfection among those in the lowest quintile of IgE level was 10.2 times more likely than among those with IgE levels in the highest quintile; conversely reinfection was 10.7 times more likely for those in the highest quintile for IgG4 against egg compared with those in the lowest quintile. These findings indicate that the gradual development of immunity to infection may, in part, depend on pathways involving IgE, some of which might be blocked by the high levels of IgG4 in younger individuals.

The participation of IgE in numerous anti-parasite effector mechanisms *in vitro* indicates that it might have a function in parasite attrition *in vivo*¹⁸. The interaction of IgE through specific receptors with eosinophils, macrophages and platelets, and the ability of these inflammatory cells to kill schistosomes *in vitro*, has been established in experimental models¹⁸. Cross-linking of IgE bound to the surface of mast cells and the subsequent release of chemotactic agents such as leukotriene-B₄, platelet-activating factor and cytokines allow rapid amplification of these responses. The association of high levels of specific anti-worm IgE with low levels of reinfection implies that these mechanisms may be important in man.

Significant amounts of parasite-specific IgG4 are found not only in schistosomiasis¹³ but also in some other helminth infections¹⁹, but its function is not clear. IgG4 is inefficient at activating complement and in binding to receptors on monocytes and macrophages; it may interfere with complement activation by IgG1 and also block mast-cell degranulation by competing with specific anti-parasite IgE for allergenic worm antigens^{13,14}.

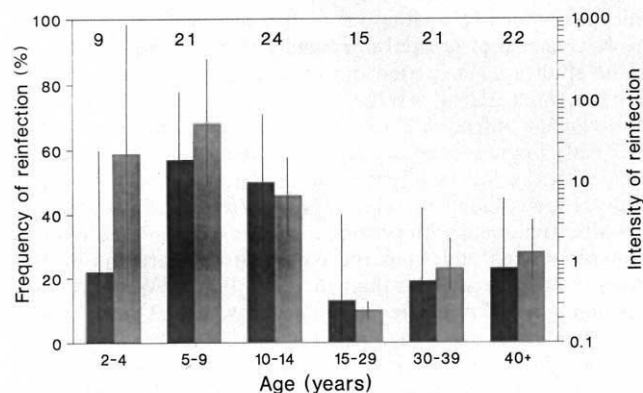


FIG. 1 Frequency (dark hatching) and intensity (light hatching) of reinfection with *S. haematobium* 15 months after chemotherapy with praziquantel in a cohort drawn from the village of Medina, Upper River Division, The Gambia. Intensity of reinfection is plotted as the geometric mean egg count of those reinfected. Numbers in each group are shown above the bars. Vertical lines represent 95% confidence intervals.

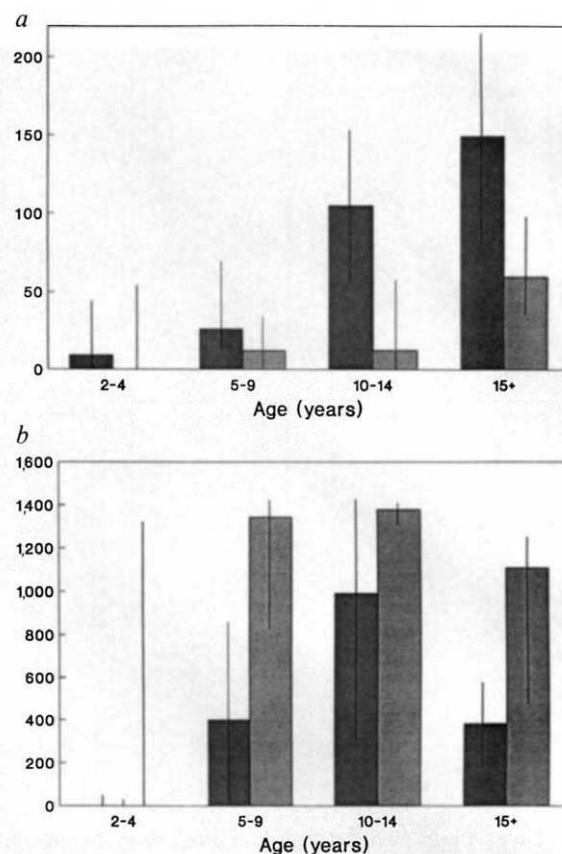


FIG. 2 a, IgE and b, IgG4 specific antibody responses to worm (dark hatching) and egg (light hatching) antigens of *S. haematobium* as determined by enzyme-linked immunosorbent assay (ELISA). Median values (absorbance at 492 nm) by age group are shown. Vertical lines represent 95% confidence intervals. For ELISA each antigen was diluted in 0.05 M Na₂CO₃ buffer, pH 9.6, and coated onto flat-bottomed microtitre plates at an optimum concentration determined by checkerboard titration using pooled positive and negative control sera. The amount of IgG subclass specific antibody bound by these antigens was as described²⁸. The amount of specific IgE bound by these antigens was determined using a mouse monoclonal anti-human IgE²⁹. Assays were developed using a horseradish peroxidase-conjugated rabbit anti-mouse IgG (Unipath, UK). Optimum concentrations of all reagents were determined by titration.

TABLE 1 Reinfection study design used in The Gambia

January/February	April/May	August-November	April/May
Pre-treatment intensity	Efficacy of treatment	Transmission Water contact observations	Reinfection intensity
Chemotherapy			

Details of the study cohort, drug treatment and methods used during observations of water contact, transmission and the index of exposure to infection are available elsewhere^{16,17}. The study was carried out in the adjacent small villages of Medina Samaco and Njarinjufa in Upper River Division, in The Gambia. Enumerated subjects were stratified by age and sex and a random sample of 40% of subjects drawn from each age/sex group. After collection of initial samples of urine for egg count studies and collection of blood for serum preparation, all the subjects in the cohort were treated with praziquantel using a single dose of 40 mg kg⁻¹ administered in the presence of H. A. Wilkins. Urine specimens were collected 3 months later to assess the effect of treatment; during this period exposure to infection was impossible as the transmission sites were dry. Five months after the end of the transmission period, urine specimens were collected to determine the intensity of reinfection. Blood samples for serum preparation were also taken three months after treatment and after reinfection. The design of this study and its subsequent conduct had the approval of The Gambia Government and MRC Ethical Committee.

TABLE 2 Logistic regression analysis

Antibody	Slope	s.e.	P	Odds of reinfection, lowest versus highest quintile*
Worm IgE	-0.580	0.252	0.02	10.2 (1.4-76.9)
Egg IgE	-0.402	0.193	0.04	5.0 (1.1-23.8)
Worm IgG4	0.557	0.229	0.02	0.1 (0.01-0.66)
Egg IgG4	0.593	0.241	0.01	0.09 (0.01-0.62)

* 95% confidence interval shown in parentheses.

Logistic regression analysis relating the risk of reinfection to levels of specific antibodies. In all models allowance has been made for cercarial exposure (4-level factor), and age and sex (5-level factor: 2-4, 5-9, 10-14, 15+ males, 15+ females). Specific antibody levels were analysed as linear effects across quintile groups based on distributions of absorbance values. In the evaluation of IgE effects, allowance has also been made for IgG4 against worm and egg antigens; in the evaluation of IgG4 effects, allowance has also been made for IgE against worm and egg antigens. Pre-treatment antibody levels were used in these analyses. Values obtained in enzyme-linked immunosorbent assay with post-treatment serum samples were closely correlated with those from pre-treatment samples. Post-treatment values gave similar results in logistic regression analyses, but because of a smaller sample size were not significant. None of the other IgG subclasses gave significant results. No evidence was found for any blocking effects of specific IgM antibody responses against worm or egg antigens (results not shown)³⁰.

Parallel recognition of filarial antigens by antibodies in immunoblots of serum from patients with filarial infection has been reported, suggesting that IgE and IgG4 might compete for the same antigenic sites²⁰. There is additional epidemiological evidence linking IgG4 with susceptibility to infection: IgG4 levels in the serum of infected Kenyan schoolchildren against the recombinant P28 glutathione-S-transferase of *S. mansoni* were found to be significantly greater in a susceptible group than in a resistant group²¹.

IgE production by human peripheral blood leukocytes is dependent on interleukin-4 (IL-4)^{22,23}. IL-4 will also stimulate IgG4 production but has no detectable effect on the synthesis of other IgG subclasses. The IL-4-dependent synthesis of both IgE and IgG4 is blocked by interferon- γ (IFN- γ)^{23,24}, although the level of IFN- γ necessary to inhibit IL-4-induced synthesis of IgE is lower than that required to stop synthesis of IgG4. The early production of specific IgG4 and later production of specific IgE could be indicative of immunoglobulin class switching caused by the sequential involvement of different lymphokines. This raises the possibility that development of protection against schistosomes and other helminth infections may depend on changes in the lymphocyte populations involved in cytokine production.

Our results do not exclude the possibility that other immunological mechanisms associated with parasite attrition could be active in young children soon after their first exposure to infection²⁵⁻²⁷. They are consistent with the hypothesis that human IgE protects against infection with *S. haematobium* and that the slow achievement of epidemiologically significant immunity reflects a delayed development of the protective IgE response and early production of IgG4 against worm and egg antigens which blocks the activity of anti-parasite IgE. □

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Programmed cell death and extrathymic reduction of $V\beta 8^+$ $CD4^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B

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CLONAL deletion and functional inactivation of self-reactive cells have been invoked as mechanisms underlying intrathymic development of T-cell tolerance¹⁻¹⁰. The relative importance of these mechanisms in the development of tolerance of more mature, peripheral T cells either to self or to exogenous antigens is unclear, although recent data relate the development of T-cell tolerance in the periphery to clonal anergy¹¹. We have now investigated the induction of extrathymic tolerance using BALB/c mice that were made tolerant to *Staphylococcus aureus* enterotoxin B (ref. 12), a superantigen which specifically interacts in such mice with T cells bearing $V\beta 8$ antigen receptors¹³⁻¹⁶. Both euthymic and athymic mice made tolerant to *S. aureus* enterotoxin B had a markedly reduced number of $V\beta 8.1,2^+$ $CD4^+$ peripheral T cells. This reduction was accompanied by genomic DNA fragmentation that is associated with cell death. These results indicate that a deletional mechanism can contribute to the induction of T-cell tolerance in peripheral lymphoid cells.

We determined the proportion of $V\beta 8.1,2^+$ T cells in the peripheral T-cell population of BALB/c mice that had been made tolerant to *S. aureus* enterotoxin B (SEB) by intravenous immunization with 50 μ g SEB. Before injection of SEB the frequency among unmanipulated splenic cells of $V\beta 8.1,2^+$ T cells bearing either $CD4$ or $CD8$ antigen was 5% and 3% respectively. Such $V\beta 8.1,2^+$ cells represented 20% of $CD4^+$ and 30% of $CD8^+$ splenic T cells. Four days after injection of SEB, the frequency of $V\beta 8.1,2^+$ cells increased 1.5-fold among both $CD4^+$ and $CD8^+$ T cells; after seven days, the proportion of $V\beta 8.1,2^+$ cells among $CD4^+$ cells decreased to 10% and among $CD8^+$ cells, returned to the pre-treatment level (Fig. 1). Also at day seven there was a reduction in the overall proportion of $V\beta 8.1,2^+$

CD4⁺ T cells among splenic cells (less than 3%), whereas the proportion of V β 8.1,2⁺ CD8⁺ cells was the same as that in controls (3%) (Y.K., personal observation). This selective reduction in the number of V β 8.1,2⁺ CD4⁺ T cells was still apparent after 21 days, although after the first week the total number of splenic cells, which was initially increased in the SEB-primed mice, returned to normal.

To investigate whether the V β 8.1,2⁺ T-cell population was selectively reduced in association with SEB tolerance, splenic and lymph node (mesenteric and inguinal) T cells were compared for expression of V β 8.1,2 and V β 6, the latter being a T-cell receptor that does not interact with SEB. Fourteen days after immunization with SEB (Table 1a) these lymphoid tissues showed a decrease in the frequency of V β 8.1,2-expressing cells among CD4⁺ T cells to about one-half of that of controls, whereas the numbers of V β 8.1,2⁺ CD8⁺ T cells were unchanged. By contrast, the frequency of V β 6⁺ expression among CD4⁺ T cells in SEB-primed mice was higher than in controls. Therefore, the SEB-reactive V β 8.1,2⁺ T cells are preferentially eliminated in SEB-tolerant mice.

To determine whether intrathymic clonal deletion contributed to the reduction in V β 8.1,2⁺ T cells, the above experiments were repeated using thymectomized mice. SEB-primed athymic mice displayed a reduction in V β 8.1,2⁺ CD4⁺ T cells comparable to that observed in euthymic mice, but expressed V β 6⁺ T cells at the same frequencies as non-tolerant controls (Table 1b). These results indicate that the reduced expression of V β 8.1,2⁺ T cells in SEB-treated mice can be attributed to selection mechanisms in the peripheral lymphoid organs that are independent of the thymus.

As these data suggested that the SEB-reactive V β 8.1,2⁺ peripheral T cells were partially eliminated in the tolerant mice, splenic T cells isolated at various days after priming with SEB were analysed for DNA fragmentation, a putative marker of programmed cell death¹⁷. DNA fragmentation was detected in cells 4 days after SEB treatment, but not at significant levels on day 2 or day 7, and never in control cells (Fig. 2a). These results indicate that programmed cell death occurs for a limited period after priming with SEB and might account for the reduction in V β 8.1,2⁺ T cells. To determine whether the cells undergoing DNA fragmentation at day 4 correspond to the V β 8.1,2⁺ cells, the expression of V β 8.1,2 was examined in splenic T-cell cultures established at 2, 4 and 7 days after SEB injection. Analyses of the cell suspensions before incubation confirmed our previous findings of an initial increase in V β 8.1,2⁺ T cells on day 2 and day 4 but a subsequent decrease by day 7 (Table 2). But after incubation for 20 h at 37 °C, the proportion of V β 8.1,2⁺ CD4⁺ T cells in the cultures established at day 4 from SEB-treated mice was about one-half of that before culture. The proportion of V β 6⁺ T cells, however, increased after incubation for 20 h,

TABLE 1 Analysis of V β 8.1,2 and V β 6 expression by T cells from normal and thymectomized mice treated with SEB

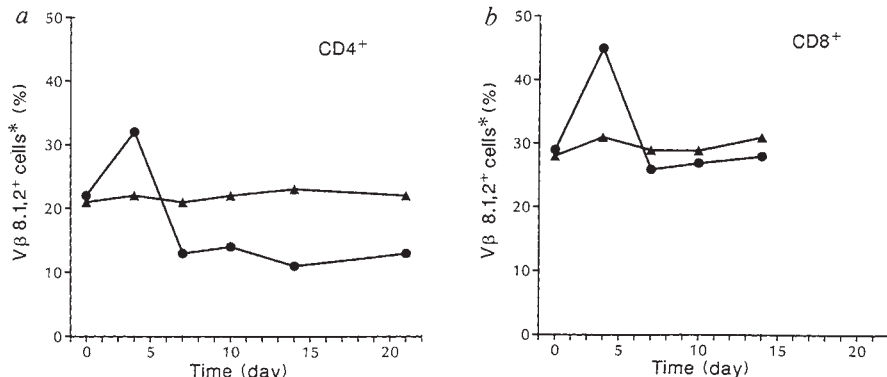
	% Positive cells			
	Spleen PBS injected	Spleen SEB injected	Lymph nodes PBS injected	Lymph nodes SEB injected
(a) Normal				
V β 8.1,2 ⁺ , CD4 ⁺ /CD4 ⁺	22.2 $\pm$ 1.8	12.9 $\pm$ 0.5	18.8 $\pm$ 1.8 (16.1 $\pm$ 0.6)	9.0 $\pm$ 1.7 (7.5 $\pm$ 1.2)
V β 8.1,2 ⁺ , CD8 ⁺ /CD8 ⁺	27.8 $\pm$ 1.7	25.5 $\pm$ 3.1	28.6 $\pm$ 0.1 (27.1 $\pm$ 1.5)	29.9 $\pm$ 0.8 (27.6 $\pm$ 3.3)
V β 6 ⁺ , CD4 ⁺ /CD4 ⁺	10.7 $\pm$ 0.5	14.6 $\pm$ 0.2	11.2 $\pm$ 0.5 (8.9 $\pm$ 1.9)	14.3 $\pm$ 1.4 (12.3 $\pm$ 1.5)
V β 6 ⁺ , CD8 ⁺ /CD8 ⁺	11.5 $\pm$ 1.0	10.7 $\pm$ 0.5	8.4 $\pm$ 0.8 (9.1 $\pm$ 2.5)	9.3 $\pm$ 1.0 (10.5 $\pm$ 1.8)
(b) Thymectomized				
V β 8.1,2 ⁺ , CD4 ⁺ /CD4 ⁺	21.4 $\pm$ 1.7	16.5 $\pm$ 0.6	18.8 $\pm$ 0.6	7.8 $\pm$ 2.6
V β 8.1,2 ⁺ , CD8 ⁺ /CD8 ⁺	26.9 $\pm$ 2.0	26.3 $\pm$ 3.6	25.2 $\pm$ 1.8	29.6 $\pm$ 2.5
V β 6 ⁺ , CD4 ⁺ /CD4 ⁺	11.3 $\pm$ 1.9	13.8 $\pm$ 0.9	11.9 $\pm$ 0.4	14.4 $\pm$ 1.5
V β 6 ⁺ , CD8 ⁺ /CD8 ⁺	9.2 $\pm$ 1.5	9.2 $\pm$ 0.5	8.4 $\pm$ 2.5	9.3 $\pm$ 0.2

Single-cell suspensions were prepared from spleen, mesenteric or inguinal (shown in parentheses) lymph node tissue 14 days after tail-vein injection of either 50 μ g SEB in 0.2 ml PBS or 0.2 ml PBS alone into normal or thymectomized 4–6-week-old BALB/c mice. The spleen cells were treated with Tris-buffered 0.16 M ammonium chloride to lyse red blood cells. The cells were then stained with either anti-V β 8.1,2 antibody (KJ16-133) or a rat IgG2b V β 6-specific antibody (RR4-7 B cell hybridoma²⁵), followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG and anti-CD4 or anti-CD8 antibody as described in Fig. 1. Two-colour fluorescence analysis was done on a Coulter Epic-C fluorocytometer. The results are presented as the mean and s.d. of triplicate samples. The results are expressed as percentage of total CD4⁺ or CD8⁺ cells dually stained by a specific anti-V β antibody and either anti-CD4 or anti-CD8 antibody, respectively.

possibly reflecting a compensation for the loss of V β 8.1,2⁺ T cells. Some reduction of the V β 8.1,2⁺ CD8⁺ T cells also occurred in these cultures, but was not greater than that in PBS-treated control cultures. By contrast, the proportion of V β 8.1,2⁺ cells in splenic cell cultures established either 2 or 7 days after priming with SEB was the same after 20 h of incubation as at the onset. We also assayed DNA fragmentation in purified V β 8.1,2⁺ and V β 6⁺ T cells of SEB-primed spleen on day 4 (Fig. 2b), and demonstrated the programmed death of V β 8⁺ but not V β 6⁺ T cells, confirming the observation of Table 2 more directly. Therefore, the reduction of V β 8.1,2⁺ T cells in SEB-primed mice coincides with and is mediated by programmed cell death triggered by stimulation with SEB.

The V β 8.1,2⁺ CD4⁺ T cells in SEB-primed mice, when cultured, fail to proliferate or produce interleukin-2 in response to stimulation by SEB¹¹. Therefore tolerance to SEB seems to be achieved not only by clonal deletion, but also by anergy of T cells expressing V β 8⁺ receptors.

FIG. 1 V β 8.1,2 expression by CD4⁺ (a) and CD8⁺ (b) T cells in SEB-primed mice. BALB/c mice (H-2^d, Mls-2^a) were given a tail-vein injection of 50 μ g SEB in 0.2 ml PBS. Spleen cells were studied by two-colour flow cytometric analysis for expression of V β 8.1,2 and CD4 (a) or CD8 (b) at the indicated times after the injection. $\blacktriangle$, PBS-primed control spleen; $\bullet$, SEB-primed spleen. Splenic single cell suspensions were treated with Tris-buffered 0.16 M ammonium chloride to lyse the red blood cells, and incubated with a rat immunoglobulin (Ig) G2a antibody to V β 8.1,2 (culture supernatant from the KJ16-133 B cell hybridoma²⁵), washed and incubated with FITC-goat anti-rat IgG. Cells were then incubated with either phycoerythrin-anti-CD4 antibody (Becton Dickinson) or with biotin-conjugated antibody to CD8 (Becton Dickinson) followed by streptavidin-phycoerythrin. Each analysis was done on 2×10^4 cells. The percentage of CD4⁺ T cells expressing V β 8.1,2 was calculated by dividing the number of cells stained with both anti-V β 8.1,2 and anti-CD4 antibodies by the total



number of cells stained with anti-CD4 antibody. The percentage of CD8⁺ T cells expressing V β 8.1,2 was calculated in the same manner. Results are presented as the arithmetic mean of triplicate samples.

TABLE 2 Analysis of V β 8.1,2 and V β 6 expression by cultured spleen cells from mice treated with SEB

Incubation	% Positive cells									
	CD4 ⁺		V β 6 ⁺		V β 8.1,2 ⁺		CD8 ⁺		V β 6 ⁺	
	PBS*	SEB*	PBS	SEB	PBS	SEB	PBS	SEB	PBS	SEB
Day 4										
Control†	20.9±1.2	28.4±1.0	10.9±0.6	9.2±0.6	29.1±2.4	43.5±1.2	9.4±0.6	4.2±0.8		
4 °C	21.1±0.5	28.1±1.6	11.4±0.9	10.2±1.1	31.4±1.4	46.0±1.3	11.3±0.3	6.5±0.5		
37 °C	21.1±1.2	15.3±2.0	12.4±0.8	13.1±1.4	31.6±1.4	33.8±1.1	11.2±1.0	11.8±1.3		
Day 2										
Control	21.5±1.0	40.7±4.1	9.8±0.3	5.8±2.0	29.9±3.5	35.3±0.5	11.5±0.3	7.0±1.1		
37 °C	19.7±1.5	36.8±2.3	10.2±0.7	4.6±0.6	29.3±1.4	37.7±2.9	12.3±1.4	8.2±1.7		
Day 7										
Control	22.9±0.5	12.2±0.2	13.2±1.6	12.2±0.6	30.0±0.7	23.9±2.8	10.5±1.7	9.8±1.1		
37 °C	22.2±2.3	8.9±1.8	12.7±1.8	14.0±0.3	29.3±1.7	24.6±1.8	12.7±1.2	12.9±2.6		

Splenic single-cell suspensions were prepared from SEB-primed BALB/c mice 2, 4 and 7 days after injection and treated with Tris-buffered 0.16 M ammonium chloride to lyse the red blood cells. Cells were enriched for T cells by depletion of surface immunoglobulin-positive cells on goat anti-mouse immunoglobulin-coated plates. The non-adherent T cells (90% Thy-1⁺) were cultured as described in Fig. 2, collected after 20 h and analysed for V β and CD phenotypes as outlined in Fig. 2 and Table 1. Data represent the mean and s.d. of three samples.

* PBS, 0.2 ml PBS injected; SEB, 0.2 ml PBS containing 50 μ g SEB injected.

† Control, cells before culture.

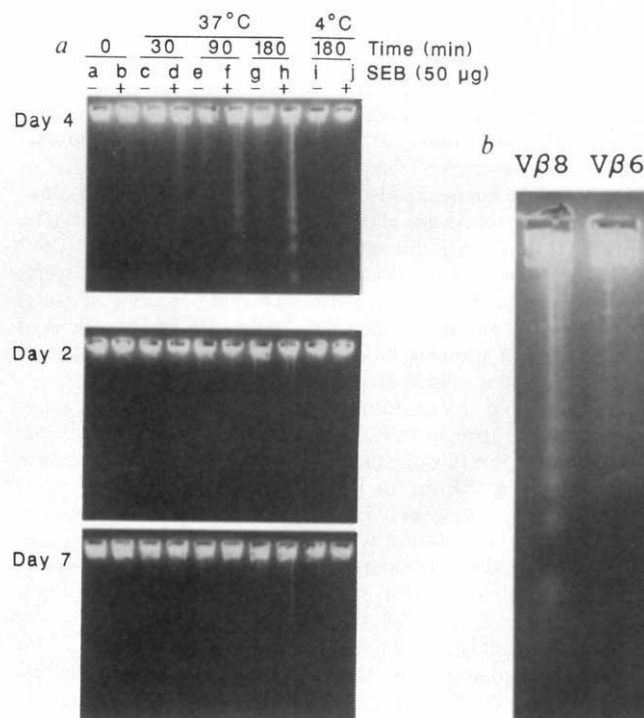
Repeated injection of neonatal mice with SEB has been previously associated with the deletion of V β 8⁺ cells in both the CD4⁺ and CD8⁺ T-cell subsets owing to negative selection in the thymus¹⁸. Our results, however, indicate that among peripheral T cells of adult mice treated with SEB, the induced extrathymic deletion of V β 8⁺ T cells occurs mainly in the CD4⁺ subset by day 7 after injection, whereas at day 2 and 4 the frequency of V β 8.1,2⁺ cells is increased. Similarly, the finding that there is expansion of V β 8⁺ T cells in lymph nodes of adult mice 3 days after priming with SEB and a deletion of V β 8⁺ T cells in the thymus¹⁷, indicates a delay in the deletion of

peripheral SEB-reactive cells after priming. This delay may reflect a relative resistance of mature, peripheral T cells to tolerance induction, as suggested by Matzinger *et al.*¹⁹, who showed that the induction of antigen-specific clonal deletion in CD4 or CD8 single-positive as well as double-positive T cells is achieved more easily in thymocytes than peripheral cells.

In contrast to the results reported here, induction of extrathymic tolerance to Mls (ref. 11) and class I or class II (refs 20–22) major histocompatibility complex alloantigens has been reported to correlate largely to functional inactivation of self-reactive cells. In those studies, however, the spectrum of

FIG. 2 DNA fragmentation in response to SEB injection. *a*, Analysis of total T cells. Fragmentation of genomic DNA from SEB (indicated as '+') and PBS (indicated as '-') treated mice was analysed in fresh spleen T cells (lanes *a* and *b*), in spleen T cells cultured at 37 °C for 30 min (*c* and *d*), 90 min (*e* and *f*) and 180 min (*g* and *h*), and in spleen T cells incubated at 4 °C for 180 min (*i* and *j*). Data shown represent results of one of five separate experiments. *b*, Analysis of purified V β 8⁺ and V β 6⁺ T cells.

METHODS. *a*, Splenic single-cell suspensions prepared 4 days after SEB or PBS (control) injection of BALB/c mice were treated with Tris-buffered 0.16 M ammonium chloride to lyse the red blood cells and enriched for T cells by depletion of surface immunoglobulin-positive cells on goat anti-mouse immunoglobulins (100 μ g ml⁻¹)-coated Petri dishes (5 × 10⁷ cells per 10-cm dish). The non-adherent T cells (90% Thy-1⁺) were cultured in 24-well culture plates at 2 × 10⁶ cells per ml in RPMI 1640 medium supplemented with 10% FCS and 5 × 10⁻⁵ M 2-mercaptoethanol. Measurements of DNA fragmentation were done as previously described¹⁶. Briefly, 2 × 10⁶ cells were washed, pelleted and resuspended in 20 μ l 10 mM EDTA, 50 mM Tris-HCl containing 0.5% sodium lauryl sarkosinate and 0.5 μ g ml⁻¹ protease K. After incubation for 1 h at 50 °C, 10 μ l 0.5 μ g ml⁻¹ RNase A was added to each sample, followed by another 1-hour incubation at 50 °C. Samples were then heated to 70 °C, mixed with 10 μ l 10 mM EDTA containing 1% low-melting-point agarose, 0.25% bromophenol blue and 40% sucrose, and electrophoresed in 80 mM Tris-phosphate/EDTA buffer over a 2% agarose gel containing 0.1 μ g ml⁻¹ ethidium bromide. Fractionated DNA was visualized by exposure to ultraviolet light. *b*, Spleen T cells were incubated with purified anti-V β 8.1,2 or anti-V β 6 antibodies at 50 μ g per 10⁷ cells on ice for 1 h. Cells are washed and further incubated for 30 min with sheep anti-rat IgG (Fc)-magnetic beads (Dynabeads M-450 Dinal Inc., Oslo, Norway). Magnetic bead-bound V β ⁺ cells are positively selected by magnet-activated cell sorter (Miltenyi Biotec GmbH, Meitzfeld, Germany). Purification for positively coated cells was performed three times, resulting in >90% purity for the V β phenotype selected. For the fragmentation of genomic DNA, 10⁶ cells studied as mentioned in methods for *a*. Data shown represent results of one of three separate experiments.



cells mediating presentation of the target antigens was restricted to, for example, B cells in the case of Mls antigen, and pancreatic cells in the presenting of major histocompatibility complex transgene products. This limitation, which would not be associated with SEB-priming, might account for the observed discrepancy, as the nature of the antigen-presenting cells involved in tolerance induction seems to greatly influence the mechanisms by which tolerance is achieved^{23,24}. In addition, the use in other studies of tolerizing antigens which, unlike SEB, represent endogenous products of the presenting cells, may restrict the

amount of antigen available for tolerance induction. In other words, as suggested above, the induction of tolerance in peripheral T cells by a deletional mechanism may occur only when there are relatively large amounts of tolerizing antigen.

The results presented here indicate that extrathymic tolerance can be achieved by both deletion and functional inactivation of antigen-reactive T cells. The degree to which these findings can be extrapolated to the induction of self-tolerance in the peripheral lymphoid system remains to be determined. □

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'Coatomer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles

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GOLGI-derived coated vesicles contain a set of coat proteins of relative molecular mass 160,000 (M_r 160K; α -COP), 110K (β -COP), 98K (γ -COP) and 61K (δ -COP), and several smaller subunits¹. We have now identified and purified a cytosolic complex containing the same four coat proteins as those of Golgi transport vesicles. We term this complex the Golgi coat promoter or 'coatomer'. The coatomer also contains polypeptides of M_r 36K, 35K and 20K. It represents about 0.2% of soluble cytosolic protein. Gel filtration of unfractionated cytosol indicates that β -COP resides exclusively in the coatomer complex. The complex seems to be a likely candidate for the unassembled precursor of Golgi coated vesicles, and its purification should help investigations of the role of coat proteins in membrane budding, for which it is necessary to use a refined cell-free system.

We discovered the coatomer during purification of an unrelated cytosolic transport factor required for *cis* to *medial* vesicular transport through the Golgi apparatus. The purification of this transport factor (to be described in detail elsewhere) used bovine brain cytosol (Fig. 1a, lane 1) as a starting material, and involved precipitation with ammonium sulphate (Fig. 1a, lane 2), followed by chromatography on DEAE-cellulose (Fig. 1a, lane 3), hydroxylapatite (Fig. 1a, lane 4) and mono-Q anion exchanger (Fig. 1a, lane 5). After mono-Q chromatography, the material was dialysed at pH 5.8 in preparation for cation-exchange chromatography. During dialysis a precipitate is formed that is removed by centrifugation and the transport activity

can be further purified from the supernatant. When the precipitate was analysed by SDS-PAGE (Fig. 1a, lane 6) we noted that the protein profile was remarkably similar to Golgi-derived 'non-clathrin'-coated transport vesicles^{1,2} containing prominent components of M_r 160, 110, 98 and 61K in highly enriched form.

The precipitate was solubilized in pH 8.0 buffer containing 1 M salt and chromatographed on a Superose-6 gel-filtration column (Fig. 1a, lane 7; Fig. 2). The product was a monodisperse complex (Fig. 2) consisting of seven polypeptides with apparent M_r s of 160, 110, 98, 61, 36, 35 and 20K on SDS-PAGE (Fig. 1b, c). A typical preparation yields ~0.5 mg coatomer from ~4 g bovine brain cytosol protein (data not shown).

Immunoblots of fractions through the ammonium sulphate precipitation, and the DEAE, hydroxylapatite and mono-Q chromatography steps probed with a monoclonal antibody recognizing the 110K coatomer component³ (see below), indicate that most but not all of the complex occurs in the fractions containing the transport factor. Therefore, that this complex copurifies with the transport factor in four steps seems to be fortuitous. The purification of the complex can be monitored by immunoblotting with the anti-110K antibody with the same results (unpublished observations).

The apparent molar ratios of the coatomer subunits, as determined by densitometry of the gels stained with Coomassie blue (Fig. 1b, c) and relative to the 160K component, were (1.0): 1.5:1.7:1.1:0.4:0.4:0.2 for the 160K-20K components, respectively. The composition and stoichiometry of the complex are highly reproducible from preparation to preparation. The complex reprecipitates at pH 5.8 and could be cycled between soluble and aggregated states at least three times (data not shown). All the subunits remained in the complex in constant proportions through these cycles.

The resolubilized coatomer elutes from gel filtration columns at an apparent M_r corresponding to a Stokes' radius of 98 Å (Fig. 2). The coatomer has a sedimentation coefficient of about 11S (data not shown). Together these data suggest⁴ that the coatomer has a native M_r of about 650-700K, and a non-globular shape.

The polypeptides of 160, 110, 98 and 61K in the coatomer comigrate in SDS-PAGE gels with corresponding Golgi-derived vesicle polypeptides (Fig. 3a). A monoclonal antibody³ that

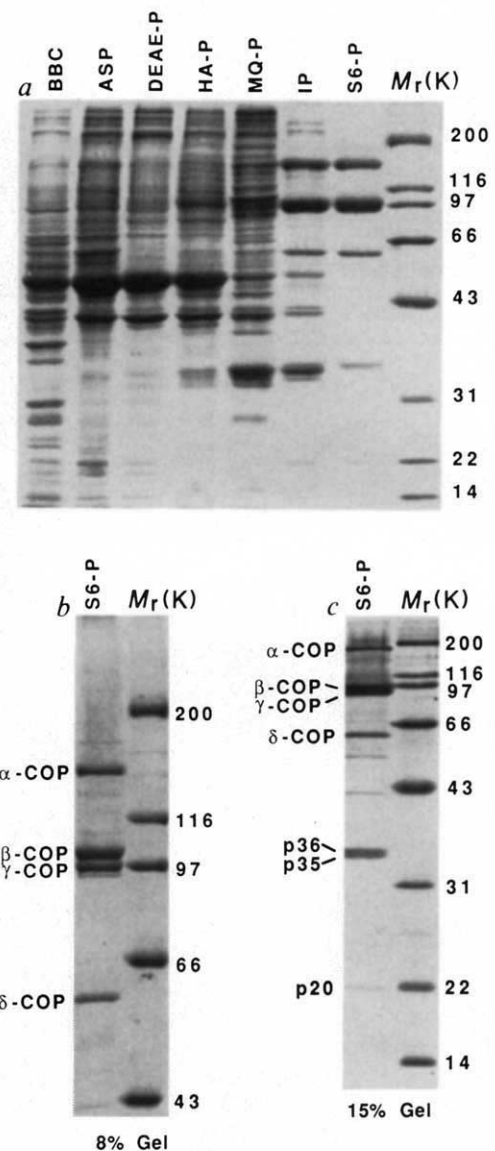
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recognizes β -COP on vesicles¹ also recognizes the 110K coatomer component (Fig. 3b). In addition, N-terminal sequence analyses (F. Wieland, personal communication) of the 160K subunits in the coatomer and on vesicles confirmed that these proteins are the same. These identities make it seem likely that the other comigrating components (such as the 98K and 61K subunits) are also authentic coated vesicle proteins. We thus refer to the coatomer polypeptides as coat proteins (COPs): α -COP (160K), β -COP (110K), γ -COP (98K) and δ -COP (61K).

The other coatomer components are termed p36, p35 and p20 on the basis of their apparent M_r s, as it is not yet certain that these are also found in coated vesicles. The p35 and p36 components do not react with anti-clathrin light-chain antibodies (data not shown), or an anti-peptide antibody that recognizes both α -SNAP and β -SNAP (ref. 5) (W. Whiteheart, personal communication).

We found that β -COP in crude cytosol (Fig. 4) is present almost exclusively in a complex of 98 Å Stokes' radius. Based on the enrichment of β -COP, the coatomer was purified about

FIG. 1 Purification of Golgi coatomer. **a**, SDS-PAGE analysis of the fractions through the purification on a 10–18% linear gradient gel. Lane 1, bovine brain cytosol (BBC, 20 μ g); lane 2, ammonium sulphate precipitate (ASP, 20 μ g); lane 3, DEAE pool (DEAE-P, 20 μ g); lane 4, hydroxylapatite pool (HA-P, 20 μ g); lane 5, mono-Q pool (MQ-P, 20 μ g); lane 6, isoelectric precipitate (IP, 10 μ g); lane 7, Superose-6 pool, that is, coatomer (S6-P, 5 μ g); lane 8, M_r markers (myosin, 200K; β -galactosidase, 116K; phosphorylase b, 97K; bovine serum albumin, 66K; ovalbumin, 43K; carbonic anhydrase, 31K; soybean trypsin inhibitor, 22K). **b**, SDS-PAGE analysis of coatomer on an 8% gel. Lane 1, coatomer (S6-P, 5 μ g); lane 2, M_r markers. **c**, SDS-PAGE analysis of coatomer on a 15% gel. Lane 1, coatomer (S6, 10 mg); lane 2, M_r markers. **METHODS.** Bovine brains were obtained immediately after slaughtering and stored in ice-cold 25 mM Tris-HCl, pH 7.4, 320 mM sucrose until use. All subsequent manipulations were done at 0–4 °C. After removal of extraneous matter, blood and meninges, 450 g tissue was homogenized twice for 30 s separated by 2 min of cooling in a Waring blender in 800–810 ml 25 mM Tris-HCl, pH 8.0, 500 mM KCl, 250 mM sucrose, 2 mM EGTA, 1 mM DTT, 1 mM PMSF, 0.5 mM 1,10-phenanthroline, 2 μ M pepstatin A, 2 μ g ml⁻¹ aprotinin, 0.5 μ g ml⁻¹ leupeptin. The homogenate was centrifuged in a Sorvall GS3 rotor at 8,500 r.p.m. (7,456 g_{avg} , k factor=2,989) for 1 h. The pooled supernatants were centrifuged at 39,000 r.p.m. in a Beckman 55Ti rotor (119,000 g_{avg} , k factor=117) for 2 h. The pooled supernatants were dialysed in Spectra/por 2 tubing (M_r cutoff 12–14K, 6.4 ml cm⁻¹) against 30 l 25 mM Tris-HCl, pH 7.4, 50 mM KCl, 1 mM DTT (50KTD; some buffers are abbreviated as follows: xK, x mM KCl; T, 25 mM Tris-HCl, pH 7.4; D, 1 mM DTT; G, 10% (w/v) glycerol). After 2-h dialysis was continued in 30 l fresh buffer for 4–12 hours. The dialysed material was clarified by centrifugation in a Sorvall GS3 rotor at 8,500 r.p.m. for 1 h. The supernatants were pooled and termed bovine brain cytosol (BBC). The concentration of the bovine brain cytosol was adjusted to 5 mg ml⁻¹ by dilution with fresh 50KTD. EDTA was added to 1 mM, and ammonium sulphate slowly added to a final concentration of 40% saturation at 0 °C. The solution was incubated for 30 min and then centrifuged in a Sorvall GS3 rotor at 8,500 r.p.m. for 1 h. The pellets were resuspended in 40 ml TDG with 10 strokes in a 40 ml Dounce homogenizer with the 'B' pestle. Using conductivity measurements, enough TDG was added to bring the final conductivity to the equivalent of 100KTDG. Insoluble material was removed by centrifugation in a Sorvall GS3 rotor at 8,500 r.p.m. for 10 min. This material is termed ASP. The ASP was loaded onto a ~700-ml DEAE cellulose (Sigma) column (2.6-cm internal diameter (ID)) equilibrated in 100KTDG at 8 ml min⁻¹ and the column washed with 300 ml equilibration buffer. The column was eluted with a 0.3 mM ml⁻¹ KCl gradient (that is, 100KTDG to 350KTDG in 833 ml). The fractions containing transport activity, usually from about 220–300 mM KCl, were pooled. This material is referred to as DEAE-P. The KCl concentration of DEAE-P was determined by measuring the conductivity, and then adjusted to 200KTDG by addition of TDG. Phosphate was added to 10 mM by addition of 200KTDG, 500 mM potassium phosphate. The solution was loaded at 1.25 ml min⁻¹ onto a 140-ml hydroxylapatite (HA Ultrogel from IBF) column (1.6-cm ID) equilibrated in 200KTDG, 10 mM potassium phosphate. The column was washed with 70 ml of the same buffer and then eluted with a 0.5 mM ml⁻¹ phosphate gradient in 200KTDG. The fractions with transport activity, usually from about 55–120 mM potassium phosphate, were pooled. This material is termed HA-P. The HA-P was diluted with TDG to the same conductivity as 150KTDG and then centrifuged in a Sorvall GS3 rotor at 8,500 r.p.m. for 10 min. The supernatant was loaded at 3 ml min⁻¹ onto an 8-ml mono-Q FPLC column (Pharmacia) equilibrated in 150KTDG. The protein was eluted with a 2.2 mM ml⁻¹ KCl gradient in TDG. The fractions containing transport activity, usually from about 340–380 mM KCl, were pooled and termed MQ-P. The MQ-P was transferred to Spectra/por 2 dialysis tubing (2 ml cm⁻¹) and dialysed extensively against 25 mM 2[(N-morpholino)ethanesulphonic acid, pH 5.8, 1 mM DTT, 10% glycerol at 4 °C. The solution was centrifuged in a Sorvall SS34 rotor at 10,000 r.p.m. (7,796 g_{avg} , k factor=3,007) for 15 min. The supernatant was aspirated and the pellet washed with fresh dialysis buffer. The pellets were resuspended



in 2 ml 25 mM Tris-HCl, pH 8.0, 1 M KCl, 1 mM DTT, 10% glycerol (resuspension buffer) and the material is referred to as IP. IP was chromatographed on Superose 6 (Pharmacia; 200 μ l on a 24 ml HR 16/10 column or 2 ml on a 100-ml Prep Grade Superose-6 column (1.6-cm ID)) in resuspension buffer, the fractions analysed by SDS-PAGE, and the fractions containing the complex of 160, 110, 98, 61, 36, 35 and 20K polypeptides (at about 10⁶K) were pooled. This material is termed S6-P or coatomer. To prepare for electrophoresis, samples were incubated at room temperature for 15 min with 0.02% deoxycholate, precipitated with 6% trichloroacetic acid at 0 °C for 1 h, and centrifuged in a microfuge for 5 min at 4 °C. The pellets were extracted with acetone at 0 °C for 15 min, centrifuged again, and finally resuspended in 12 μ l SDS-PAGE sample buffer¹⁴ by incubation at 50 °C for 15 min. SDS-PAGE was as described by Laemmli¹⁴ in a minigel format and stained with Coomassie blue R-250. Protein concentrations were determined with the BioRad protein assay kit.

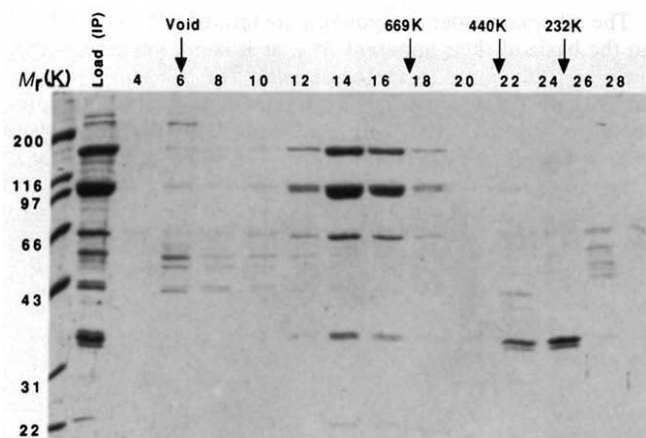


FIG. 2 Gel-filtration chromatography of coatomer. SDS-PAGE analysis on a 10–18% linear gradient gel of fractions from a Superose-6 column of resuspended isoelectric precipitate. M_r markers as in Fig. 1; IP, 10 μ g isoelectric precipitate (that is, column load); numbers, fractions from the column.

METHODS. Resuspended isoelectric precipitate (200 μ l) was chromatographed on a 24-ml Superose-6 column (1-cm ID) in resuspension buffer at 0.3 ml min⁻¹. Fractions (0.5 ml) were collected and 50 μ l aliquots were prepared for electrophoresis as described in Fig. 1 legend. M_r standards (Pharmacia) for gel filtration were bovine thyroid thyroglobulin (669K), horse spleen ferritin (440K), bovine liver catalase (232K).

500-fold from cytosol with a 7% yield, corresponding to about 0.2% of bovine brain cytosol protein. This is comparable to the content of clathrin in many different cell types⁶.

Although we lack any direct evidence for the function of the coatomer in transport, its existence suggests that it represents the cytosolic assembly unit of the coat of Golgi transport vesicles. One or more of the coatomer polypeptides could be mammalian homologues of yeast *SEC* gene products involved in vesicle budding, some of which are thought to be interacting gene products⁷.

Brefeldin A, which disrupts Golgi function^{8–10}, causes release of β -COP from the Golgi membrane into a dispersed cytosolic pool¹¹. Perhaps brefeldin A disrupts Golgi coat structure by directly interacting with β -COP or with a component that is required for the binding of the coatomer to the Golgi membrane.

There are some striking similarities between the M_r s of the Golgi coatomer polypeptides and those of clathrin-adaptor complexes, the protomers of clathrin-coated vesicles^{12,13}. Correspondences suggesting possible homologies exist for the 180K clathrin heavy chain (α -COP), the 30–40K light chains (p35 and p36), and for the subunits present in each clathrin adaptor complex: two 100–110K adaptins (β -COP and γ -COP), a 47–50K subunit (δ -COP), and a subunit of 16–20K (p20). Such homologies have a precedent in the homology of β -COP and β -adaptin (ref. 1; R. Duden *et al.*, manuscript submitted). Perhaps the clathrin and adaptor portions of clathrin coats dissociate from each other, whereas the analogous portions of Golgi coated vesicle coats stay together in a complete coatomer.

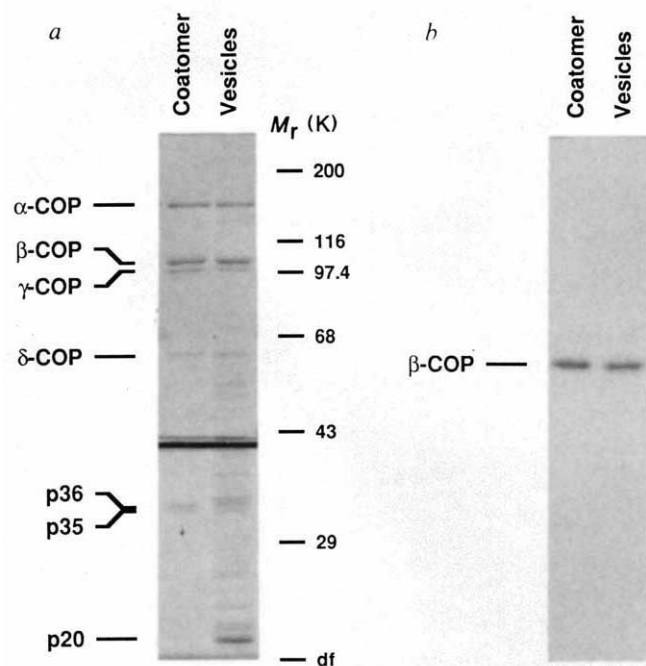


FIG. 3 Comparison of coatomer and Golgi-derived coated vesicles. *a*, SDS-PAGE analysis. In the top portion of the figure (above 43K), 0.6 μ g coatomer and about 1/17 of the peak fraction (number 8) of the final gradient of a rabbit liver Golgi-derived coated vesicle preparation¹ were separated on a 7.5% gel. In the bottom portion, 2.4 μ g coatomer and about one-third of the peak vesicle fraction were electrophoresed on a 12.5% gel; only the lower M_r portion (<43K) of the gel is shown. Abbreviation: df, dye front. *b*, Immunoblot of 0.08 μ g coatomer and 1/125 of the peak vesicle fraction electrophoresed in a 7.5% gel and probed with anti- β -COP monoclonal antibody (M3A5) (ref. 3).

METHODS. *a*, *b*, Vesicles were prepared, and 10 μ g coatomer and the peak fraction (number 8) were TCA-precipitated as described¹. *a*, M_r markers for the 7.5% gel were as in Fig. 1 legend; the 29K marker in the 12.5% gel is carbonic anhydrase from a different source. *b*, Immunoblotting using M3A5 monoclonal antibody³ to detect β -COP was performed as described¹.

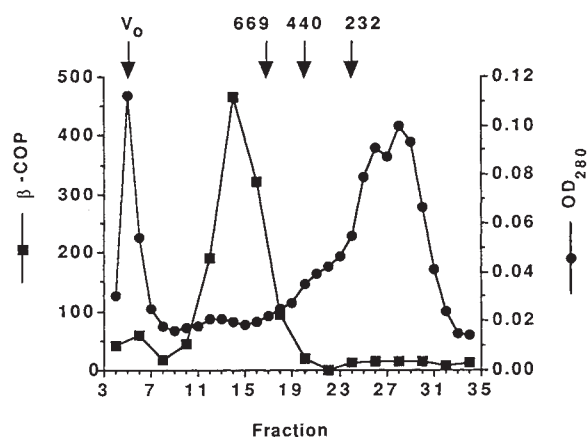


FIG. 4 Gel filtration of coatomer in cytosol. Bovine brain cytosol was gel-filtered and samples analysed by immunoblotting with monoclonal antibody M3A5 that recognizes β -COP. OD₂₈₀, optical density at 280 nm. V_0 , void volume.

METHODS. BBC (200 μ l) at 5 mg ml⁻¹ was chromatographed on a 24-ml Superose-6 column (1-cm ID) in resuspension buffer at 0.3 ml min⁻¹. Fractions of 0.4 ml were collected and 100- μ l aliquots were prepared for electrophoresis on an 8% gel as described in Fig. 1 legend. After electrophoresis the gel was soaked in transfer buffer¹⁵ for 10 min then blotted onto 0.45- μ m nitrocellulose (Schleicher and Schuell) at 100 V for 1 h in a BioRad mini-protein II transfer cell. The nitrocellulose was blocked with 5% (w/v) non-fat milk (w/v), PBS, 0.1% (w/v) Tween-20 (M/PBS/T) for 30 min, incubated in a 1:500 dilution of M3A5 monoclonal antibody in the same buffer for 1 h, washed three times for 10 min each with PBS/T, incubated in a 1:3,000 dilution of affinity-purified rabbit anti-mouse IgG (Organon Teknica-Cappel) in M/PBS/T for 1 h, washed as before, incubated in 0.2 μ Ci ml⁻¹ [¹²⁵I]protein A (ICN) in M/PBS/T for 1 h, washed as above, dried and exposed to Kodak X-AR 5 film at -80 °C with an intensifying screen for 15 h. The blot was quantitated by excision of the 110K region from the nitrocellulose and counting in a Beckman 5500B γ counter.

The ability to isolate the coatomer in milligram quantities will greatly facilitate studies of the mechanism of budding and of the structure of the coat, hampered until now by the fact that only microgram quantities could be obtained from purified Golgi coated vesicles^{1,2}. □

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In vitro effects on microtubule dynamics of purified *Xenopus* M phase-activated MAP kinase

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The protein kinase MAP kinase, also called MAP2 kinase, is a serine/threonine kinase whose activation and phosphorylation are induced by a variety of mitogens^{1–6}, and which is thought to have a critical role in a network of protein kinases in mitogenic signal transduction^{1–7}. A burst in kinase activation^{8,9} and protein phosphorylation¹⁰ may also be important in triggering the dramatic reorganization of the cell during the transition from interphase to mitosis. The interphase–metaphase transition of microtubule arrays is under the control of p34^{cdc2} kinase¹¹, a central control element in the G2–M transition of the cell cycle¹². Here we show that a *Xenopus* kinase, closely related to the mitogen-activated mammalian MAP kinase, is phosphorylated and activated during M phase of meiotic and mitotic cell cycles, and that the interphase–metaphase transition of microtubule arrays can be induced by the addition of purified *Xenopus* M phase-activated MAP kinase or mammalian mitogen-activated MAP kinase to interphase extracts *in vitro*.

Immature *Xenopus* oocytes (naturally arrested at the G2–M border in first meiotic prophase) were treated with progesterone, and kinase activities in extracts prepared from them were measured using as exogenous substrates microtubule-associated protein 2 (MAP2) and myelin basic protein (MBP), both of which are major substrates for MAP kinase^{2–6,13}. Kinase activities towards MAP2 and MBP increased after progesterone treatment and peaked just before complete germinal vesicle

breakdown (GVBD) (Fig. 1a). After completion of GVBD the kinase activities decreased and then increased again as the oocytes approached second metaphase (Fig. 1a). The activation of MAP2- and MBP-phosphorylating activities always lagged slightly behind activation of the histone H1 kinase activity, a measure of p34^{cdc2} kinase. Figure 1b shows the time course of the MAP2/MBP kinase and the H1 kinase activities following fertilization of mature oocytes. Upon fertilization (Fig. 1b) or electrical activation (data not shown) both kinase activities dropped rapidly as the eggs were released from arrest in metaphase II. The drop in the H1 kinase activity preceded that in the MAP2/MBP kinase (Fig. 1b). Both MAP2/MBP kinase and H1 kinase activities increased again as the eggs approached the first mitotic metaphase. Among the several MBP-phosphorylating activities, a major MBP kinase with an apparent relative molecular mass of 42,000 (M_r 42K) was prominently inactivated after electrical activation of mature oocytes (Fig. 1c, arrowhead), as revealed by detection of kinase activities in polyacrylamide gels containing MBP after SDS-PAGE of the extracts.

We purified the 42K M phase-activated kinase from extracts of unfertilized eggs by sequential chromatography (Fig. 2a, and

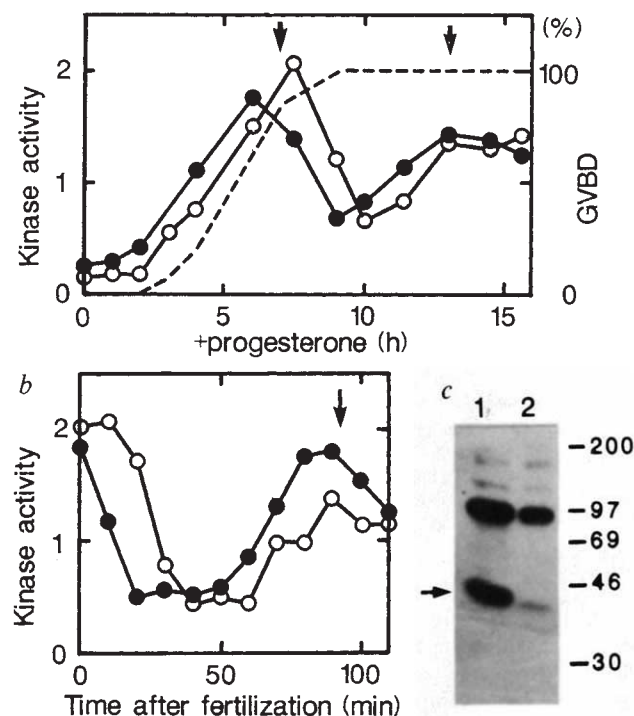


FIG. 1 Time course of *Xenopus* MAP kinase activity during oocyte maturation and following fertilization or activation of mature oocytes. *a*, Immature *Xenopus* oocytes (stage VI) were treated with 10 μ M progesterone for various times. *Xenopus* mature oocytes were *b*, fertilized, or *c*, activated by an electric shock. Kinase activities in the extracts toward MAP2 (55 μ g ml⁻¹), MBP (240 μ g ml⁻¹), and histone H1 (300 μ g ml⁻¹) were determined (*a*, *b*). Data are shown in arbitrary units (1 unit is 0.16 nmol min⁻¹ mg⁻¹ for MBP kinase and 0.02 nmol min⁻¹ mg⁻¹ for H1 kinase). The time course of MAP2 kinase activity was essentially the same as that of MBP kinase activity, and is not shown here for the sake of clarity. The broken line indicates the rate of GVBD. Arrows in *a* show the approximate timing of the first and second M phase. An arrow in *b* shows the time of first cell division. In *c* the kinase activity was detected by means of a kinase reaction in a polyacrylamide gel containing 0.5 mg ml⁻¹ MBP following denaturation and renaturation after SDS-PAGE of extracts from mature oocytes (lane 1) or activated eggs (40 min after an electric shock, lane 2).

METHODS. Oocytes were homogenized in two volumes of 60 mM β -glycerophosphate, 10 mM EGTA, 10 mM MgCl₂, 1 mM DTT, 0.1 mM NaF, 1 mM Na₃VO₄, 10 μ g ml⁻¹ leupeptin, 1% aprotinin, 0.1 mM PMSF, and 20 mM Tris-HCl pH 7.5, and extracts were obtained by centrifugation first at 5,000g for 5 min and then at 300,000g for 30 min. Kinase assays were carried out as described²⁰. The kinase assay in polyacrylamide gel was carried out according to the method of Kameshita and Fujisawa²¹.

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TABLE 1 Purification and properties of MAP kinase

Purification of M phase-activated <i>Xenopus</i> MAP kinase					
	Total protein (mg)	Total activity (nmol min ⁻¹)	Specific activity (nmol min ⁻¹ mg ⁻¹)	Recovery (%)	Purification (fold)
Crude extract	549	58	0.106	100	1
DEAE-cellulose	72.5	53	0.731	91.4	6.9
Phenyl-Sepharose	18.8	50	2.65	86.2	25.0
Heparin-Sepharose	2.4	21.6	9.00	37.2	84.9
Polylysine-agarose	1.06	21.3	20.1	36.7	190
Mono-Q	0.041	13.3	324	22.9	3,057
Properties of MAP kinases					
		M phase-activated <i>Xenopus</i> MAP kinase		Mitogen-activated mammalian MAP kinase	
Substrate* specificity (%)	Substrate				
	MAP2	100	100		
	MBP	58	47		
	histone H1	0.4	0		
	histone H2A	0.1	1.1		
	Casein	3.6	2.1		
	phosphorylase b	0	0.1		
	tau	2.9	5.1		
	tubulin	0.2	0.4		
Optimal conditions	actin	0	0.4		
	pH	8	8		
	Mg ²⁺	20 mM	20 mM		
Inhibitors (IC ₅₀)	Mn ²⁺	2 mM	2 mM		
	Zn ²⁺	0.1 mM	0.1 mM		
	Ca ²⁺	>10 mM	>10 mM		
	β-glycerophosphate	150 mM	200 mM		
	PKI†	>0.1 mg ml ⁻¹	>0.1 mg ml ⁻¹		
	K252a	0.05 μM	0.05 μM		
<i>M_r</i> on gel filtration	staurosporine	>0.5 μM	>0.5 μM		
		40–45K	40–45K		

MBP was used as a substrate in all the data shown except for determinations of substrate specificity. Essentially the same results were obtained when MAP2 was used, except that the optimal pH for MAP2 was 7.0–7.5 for both MAP kinases.

* MAP2, tau and tubulin were purified from porcine brains²⁴. Actin was from rabbit skeletal muscle. MBP, histones H1 and H2A, phosphorylase b and casein were purchased from Sigma.

† PKI, cyclic AMP-dependent protein kinase inhibitor peptide (rabbit sequence, Sigma). K252a (*Nocardia*)¹⁸ and staurosporine (*Streptomyces*)¹⁹ were purchased from Kyowa, Tokyo. Kinase assays were performed as described previously²⁰. Mammalian mitogen-activated MAP kinase was purified from rat 3Y1 cells treated with epidermal growth factor as described²⁰. We use the term 'MAP kinase' as it stands for microtubule-associated protein 2 kinase or mitogen-activated protein kinase. As MAP kinase has been shown to be activated in M phase in this study, it refers to M phase-activated protein kinase as well. IC₅₀, 50% inhibitory concentration.

Table 1). The pattern for the final chromatography step on Mono-Q is shown in Fig. 2b. The elution of a 42K polypeptide, the only detectable band on silver-stained SDS-polyacrylamide gel, coincided completely with that of the MAP2/MBP-phosphorylating activity. Kinase assays in MBP-containing polyacrylamide gels after SDS-PAGE confirmed that the 42K polypeptide corresponds to the MAP2/MBP kinase (Fig. 2b).

The properties of both the *Xenopus* 42 K kinase and the mammalian MAP kinase were examined in detail. The results clearly show the close similarity between both kinases in chromatographic behaviour (data not shown), substrate specificity, apparent *M_r* and other properties (Table 1). In addition, the patterns of phosphopeptide fragments generated by partial chymotryptic digestion of MAP2 phosphorylated by either kinase were the same. Furthermore, the partial amino-acid sequences of two of the peptide fragments obtained by lysyl endopeptidase-digestion of the purified *Xenopus* 42K kinase (data not shown) were the same as the corresponding sequences of insulin-stimulated MAP kinase¹⁴. On the basis of these results, the 42K *Xenopus* kinase is identified as a homologue of mammalian mitogen-activated MAP kinase and can be termed *Xenopus* M phase-activated MAP kinase.

The purified *Xenopus* MAP kinase was almost completely inactivated by treatment with protein phosphatase 2A (data not shown), indicating that phosphorylation of M phase-activated MAP kinase is required for its activity. To examine the phos-

phorylation state of the kinase *in vivo*, extracts of ³²P-labelled oocytes and eggs were sequentially chromatographed on DEAE-cellulose and phenyl-Sepharose, because the 42K polypeptide of activated MAP kinase was clearly visible as a discrete band in SDS gels after these steps, as seen in Fig. 2a, lane 3. The 42 K band was detected only in the MAP kinase activity fraction during subsequent purification steps, and protein bands with the same *M_r* were not detected in other fractions as analysed by SDS-PAGE with silver-staining. These findings suggest that the 42 K band in the phenyl-Sepharose fraction is the active MAP kinase. Figure 2c shows that the amount of phosphorylated kinase polypeptide increased as *Xenopus* oocytes entered meiosis I after progesterone treatment and that the phosphorylated MAP kinase at metaphase II was greatly decreased after electrical activation of mature oocytes. The phosphorylation of MAP kinase occurred on both seryl and tyrosyl residues (Fig. 2e). In agreement with this result, purified M phase-activated *Xenopus* MAP kinase was recognized by a monoclonal anti-phosphotyrosine antibody (Fig. 2d). We cannot completely rule out the possibility, however, that our phosphoamino acid analysis partly reflects phosphorylation of a minor contaminating band of the same *M_r*. In any event, phosphorylation of M phase-activated MAP kinase correlates with its activation as a kinase. The M phase-activated MAP kinase might correspond to the 42 K phosphotyrosine-containing protein¹⁵ that is detected in *Xenopus* mature oocytes. It was reported that mammalian

mitogen-activated MAP kinase is only active when both tyrosyl and threonyl residues are phosphorylated¹³. Further studies are needed to elucidate the relationship between phosphoamino acid content and the kinase activity of M phase-activated MAP kinase.

During the transition from interphase to mitosis there is a dramatic reorganization of microtubules. Using *Xenopus* egg extracts and isolated centrosomes, Karsenti and co-workers showed that the interphase-metaphase transition of microtubule arrays is under the control of the p34^{cdc2} kinase¹¹. Because active

cdc2 kinase or maturation (or M phase) promoting factor (MPF) can elicit several events of M phase even in cell-free extracts of *Xenopus* eggs, it is possible that a kinase dependent on the p34^{cdc2} kinase/MPF is involved in changes in microtubule organization. We confirmed the observation of Karsenti and co-workers that microtubules nucleated by centrosomes in interphase extracts grow faster and are longer at steady-state than those in metaphase II extracts (Fig. 3a, b, e, f). Incubation of interphase extracts with the purified M phase-activated MAP kinase resulted in a dramatic change in microtubule dynamics.

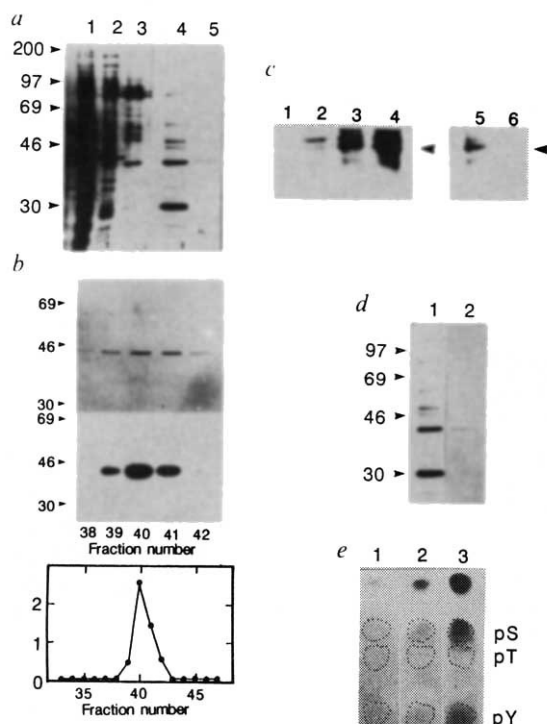


FIG. 2 Purification and characterization of *Xenopus* M phase-activated MAP kinase. **a**, SDS-PAGE of each purification step. 1, Extracts; 2, DEAE-cellulose; 3, phenyl-Sepharose; 4, polylysine-agarose; and 5, Mono-Q fractions. **b**, Chromatography on Mono-Q. Silver-staining (top), kinase assay in MBP-containing polyacrylamide gel after SDS-PAGE (middle), and elution profile of MBP kinase activity (1 unit is $0.1 \text{ nmol min}^{-1} \text{ ml}^{-1}$) (bottom). The peak fraction (no. 40) was eluted at $\sim 0.3 \text{ M NaCl}$. **c**, Phosphorylation of the kinase in M phase. Oocytes were prelabelled with $0.3 \text{ mCi ml}^{-1} \text{ }^{32}\text{P}$ -orthophosphate for 4 h in OR-2 buffer²². Immature oocytes (stage VI) were treated with $10 \mu\text{M}$ progesterone for 0 (lane 1), 2 (lane 2), 6 (lane 3) or 8 (lane 4) h. Mature oocytes were electrically activated. Lane 5, before activation; lane 6, 40 min after activation. Labelled oocytes were homogenized and sequentially chromatographed on DEAE-cellulose and phenyl-Sepharose, and analysed by SDS-PAGE and autoradiography. Arrowheads indicate the position of the 42K kinase polypeptide identified by protein staining. **d**, Immunoblotting of the partially purified M phase-activated *Xenopus* MAP kinase (polylysine fraction) with anti-phosphotyrosine antibody (PY20, ICN). 1, Silver staining; 2, immunoblotting. **e**, Phosphoamino acid analysis. M phase-activated MAP kinase from ^{32}P -labelled oocytes was excised from gels shown in **c** and analysed. Lanes 1, 2 and 3 correspond to lanes 1, 2 and 4 in **c**, respectively.

METHODS. The 42K *Xenopus* kinase (M phase-activated MAP kinase) was purified from extracts of mature *Xenopus* oocytes by sequential chromatography on DEAE-cellulose, phenyl-Sepharose, heparin-Sepharose, polylysine-agarose and Mono-Q. The buffers used were the same as those used for purification of mammalian MAP kinase²⁰. In the Mono-Q chromatography, the adsorbed proteins were eluted with a linear gradient of $0\text{--}0.8 \text{ M NaCl}$ in the buffer used for polylysine chromatography. Extracts were obtained as described in Fig. 1. The kinase was purified $\sim 3,000$ -fold and the specific activity of the purified kinase was $324 \text{ nmol min}^{-1} \text{ mg}^{-1}$ (see Table 1). Immunoblotting was carried out by the standard method with Konic Immunostaining Kit IS-50B. A relatively large amount of the 42K band ($\sim 0.5 \mu\text{g}$) on the blot was required for a positive reaction.

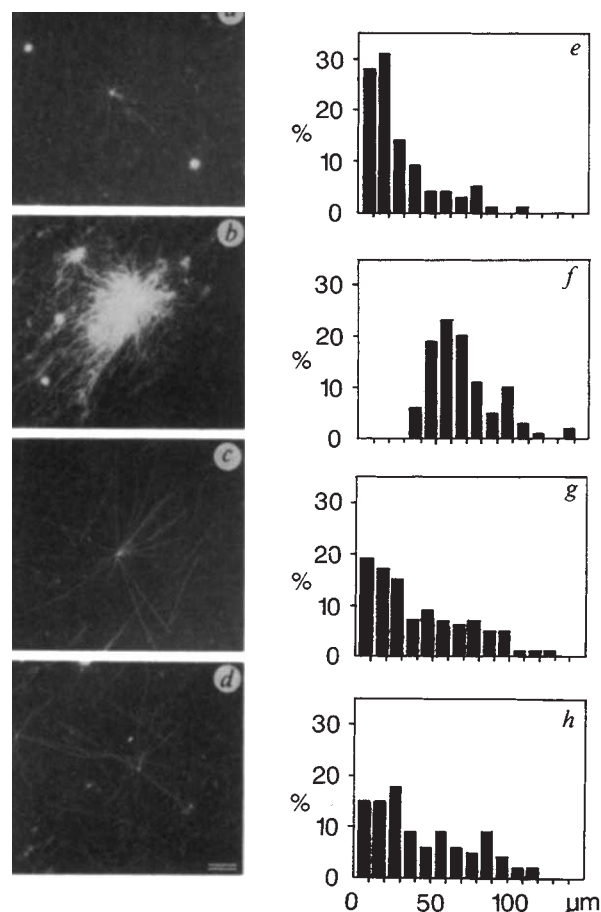


FIG. 3 Effect of MAP kinase on arrays of centrosome-nucleated microtubules in *Xenopus* interphase extracts. Purified centrosomes were added to metaphase II extracts (**a**, **e**) or interphase extracts incubated without (**b**, **f**) or with (**c**, **g**) purified *Xenopus* M phase-activated MAP kinase (Mono-Q fraction, final concentration $1.6 \mu\text{g ml}^{-1}$, the final kinase activity corresponding to about 50% of that found in the M phase extract) or with partially purified mammalian mitogen-activated MAP kinase (**d**, **h**, final MBP kinase activity about one third of that of **c**) and the reaction mixture incubated for 10 min at 20°C . Microtubules were visualized by immunofluorescence using anti- α -tubulin antibody after fixation as described²³ (**a**–**d**). The histograms show the microtubule length distribution (**e**–**h**).

METHODS. *Xenopus* extracts were prepared by a modification of the method of Lohka and Maller^{24,25}. Protein concentration was $\sim 30 \text{ mg ml}^{-1}$. As the method for preparing extracts is significantly different from that in Fig. 1, the specific MAP kinase activity (toward MBP) of metaphase II extracts was low ($0.033 \text{ nmol min}^{-1} \text{ mg}^{-1}$, or $\sim 1.0 \text{ nmol min}^{-1} \text{ ml}^{-1}$ of extract) under our standard conditions. The amount of purified *Xenopus* kinase added to the interphase extracts was $1.6 \mu\text{g ml}^{-1}$ (specific activity of $324 \text{ nmol min}^{-1} \text{ mg}^{-1}$, or $0.52 \text{ nmol min}^{-1} \text{ ml}^{-1}$ of extract), which corresponds to $\sim 50\%$ of that in the metaphase II extracts. Interphase extracts were prepared from eggs sampled 40 min after activation by an electric shock (12 V a.c. , 3 s) in MMR buffer (0.1 M NaCl , 2 mM KCl , 1 mM MgCl_2 , 2 mM CaCl_2 , 0.1 mM EDTA , 5 mM HEPES , pH 7.8). Centrosomes were prepared from CHO cells as described²⁶. Mammalian mitogen-activated MAP kinase was purified from EGF (30 nM , 5 min)-treated rat fibroblastic 3Y1 cells by sequential chromatography on DEAE-cellulose, phenyl-Sepharose, hydroxylapatite, heparin-Sepharose and polylysine-agarose²⁰ (see Table 1).

The initial apparent growing speed of microtubules decreased from 16.5 to 9.5 $\mu\text{m min}^{-1}$ and the steady-state length shortened (Fig. 3c, g) to a level similar to that in metaphase II extracts. This effect of M phase-activated MAP kinase was not seen when K252a, which strongly inhibited MAP kinase activity (Table 1), was added with MAP kinase (data not shown). This indicates that the MAP kinase-induced transition of microtubule arrays is mediated by MAP kinase-catalysed phosphorylation events. In addition, we found that mammalian MAP kinase purified from fibroblastic cells treated with epidermal growth factor, like

M phase-activated MAP kinase, caused the interphase-metaphase transition of microtubule arrays when added to the *Xenopus* interphase extracts (Fig. 3d, h). This result strengthens the functional similarity between the mitogen-activated mammalian MAP kinase and the M phase-activated *Xenopus* MAP kinase. The targets of MAP kinase in this *in vitro* reconstituted system have not been examined, but possible candidates include MAPs (for example, X-MAP, ref. 16) and S6 kinase II, known to be activated by phosphorylation on injection of MPF¹⁷ and by mammalian MAP kinase *in vitro*⁷. □

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Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18)

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FOLLICULAR lymphoma, the most common human lymphoma, characteristically has a t(14; 18) interchromosomal translocation^{1,2}. It is typically an indolent disease comprised of small resting B cells, but frequently develops into a high-grade lymphoma³. The t(14; 18) translocates the *Bcl-2* gene, generating a deregulated *Bcl-2*-immunoglobulin fusion gene⁴⁻⁸. *Bcl-2* is a novel inner mitochondrial membrane protein⁹ that extends the survival of certain cells by blocking programmed cell death⁹⁻¹¹. To determine the oncogenic potential of the t(14; 18) translocation, we produced transgenic mice bearing a *Bcl-2*-immunoglobulin minigene that structurally mimicked the t(14; 18) (ref. 12). An indolent follicular hyperplasia in these transgenic mice progressed to a malignant diffuse large-cell lymphoma. The long latency, progression from polyclonal to monoclonal disease, and histological conversion, are all suggestive of secondary changes. Half of the immunoblastic high-grade lymphomas had a rearranged *c-myc* gene. Our transgenic mice provide an animal model for tumour progression in t(14; 18) lymphoma and show that prolonged B-cell life increases tumour incidence.

Transgenic mice from all six *Bcl-2*-Ig founder lines were initially healthy but showed a 3-4 fold expansion of their IgM/IgD follicular centre B lymphocytes which manifested as benign lymphoid hyperplasia^{12,13}. Subsequently we found 18 cases of lymphoma in five of the lines. High-grade diffuse large-cell immunoblastic lymphomas (DHL-I) (ref. 14) were the most common (Fig. 1a; Table 1). All 13 cases presented as rapidly expanding abdominal masses; mesenteric lymph nodes were invariably the primary site of tumour involvement. One case of diffuse large-cell, noncleaved lymphoma, however, presented as an enlarged anterior cervical lymph node in a transgenic mouse (14/11/15) (Table 1). DHLs were disseminated in 10 out of 15 cases. A high mitotic rate was characteristic of the

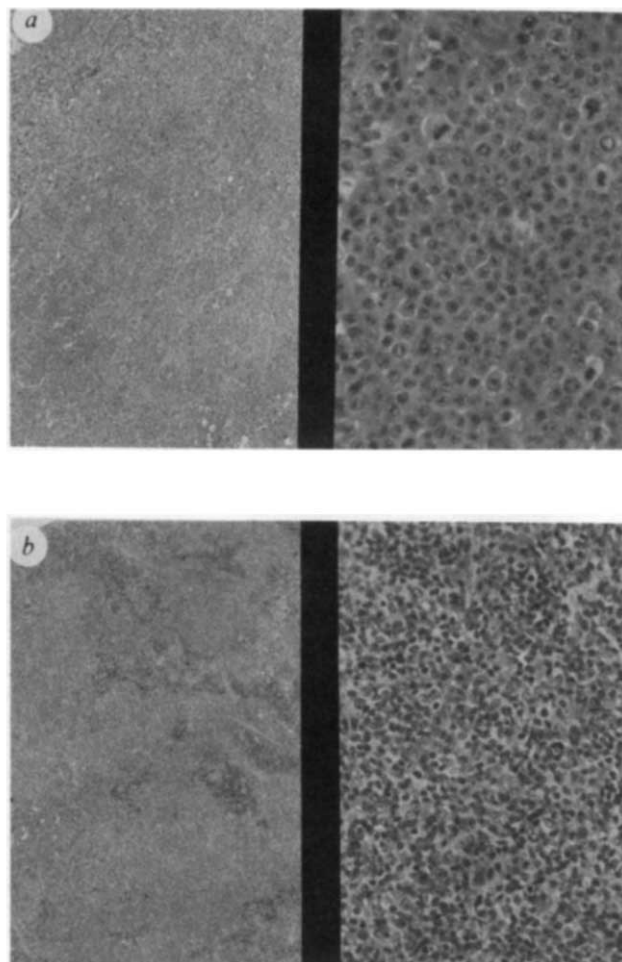


FIG. 1 Tissue sections stained with haematoxylin and eosin at low (left) and high (right) magnifications of malignant lymphomas. *a*, Representative section of mesenteric lymph node, often 2-3 cm in diameter, from transgenic mouse 59/7/1, showing complete effacement of architecture by immunoblastic lymphoma. Note numerous mitotic figures. *b*, Section of spleen from transgenic mouse 14/9 showing loss of white pulp architecture and red pulp invasion by a monomorphic population of small lymphocytes with cleaved nuclei characteristic of PDLL.

TABLE 1 Characteristics of malignant lymphomas

Mouse	Tumour watch*	Age (months)†	Transgene‡	J _H §	myc	Tumour type¶	Primary site*
14/11/10	+	14	+	C	—	DHL-I	MLN
23/11/55	+	16	+	C	—	DHL-I	MLN
23/11/75	—	10	+	C	—	DHL-I	MLN**
23/11/35/2/8	—	8	+	C	—	DHL-I	MLN
23/11/35	—	12	+	ND	+	DHL-I	MLN
43/119	—	10	+	C	+	DHL-I	MLN**
43/120	—	13	+	C	—	DHL-I	MLN**
59/7/58	—	7	+	C	+	DHL-I	MLN
59/7/18	—	15	+	C	—	DHL-I	MLN
59/7/43	+	16	+	C	+	DHL-I	MLN
59/7/1	—	16	+	C	+	DHL-I	MLN
59/7/28	—	16	+	C	+	DHL-I	MLN
59/7/23	—	16	+	ND	+	DHL-I	MLN
14/11/15	+	18	+	C	—	DHL-LNC	CLN
43/53	—	21	+	C	—	DHL-LNC	Spleen
14/9	+	18	+	O	—	PDLL	Spleen
23/18	+	18	+	O	—	PDLL	Spleen
23/11/69	—	9	+	O	—	PDLL	Spleen
43/62	—	18	—	C	—	ML-LC	Spleen
59/7/47	+	19	—	C	—	ML-LNC	Spleen

* Mice present in the tumour watch (+) were used to estimate tumour incidence (see text). Founder line is designated by the first number in the series for each mouse.

† Age at time of sacrifice.

‡ Transgene status was determined by Southern blotting of *Bam*HI-digested tail DNA using a 0.55-kb *Sac*I-digested human *Bcl-2* fragment.

§ Immunoglobulin gene heavy-chain rearrangements. C, clonal rearrangement present; O, oligoclonal rearrangement present; ND, not determined.

|| Rearrangements of *c-myc*.

¶ DHL-I, diffuse histiocytic lymphoma, immunoblastic; DHL-LNC, diffuse, large-cell, non-cleaved lymphoma; PDLL, poorly differentiated lymphocytic lymphoma. Malignant lymphoma, large cleaved (ML-LC) or non-cleaved (ML-LNC).

* MLN, mesenteric lymph node; CLN, cervical lymph node.

** Three tumours arising from a cohort of 5 transgenic mice hyperimmunized intraperitoneally with 50 µg fluorescein isothiocyanate-keyhole limpet haemocyanin every two weeks. No tumours were found in control littermates. Immunoperoxidase staining of tumour sections with 6C8 anti-human *Bcl-2* monoclonal antibody revealed *Bcl-2* protein of transgene origin in both the PDLLs and DHLs of *Bcl-2*-Ig mice (data not shown).

immunoblastic lymphomas (Fig. 1a). Flow cytometric analysis of propidium iodide-stained single-cell suspensions¹⁵ revealed that, on average, 18% of immunoblastic lymphoma cells were within the S, G2 and M phases of the cell cycle, compared with 4% of mesenteric lymphocytes from *Bcl-2*-Ig transgenic mice without tumours. Moreover, analysis of endogenous immunoglobulin gene configuration revealed that the DHLs were uniformly monoclonal B-cell malignancies (Fig. 2; Table 1).

Three transgenic mice presented with massive splenomegaly due to involvement by poorly differentiated lymphocytic lymphoma (PDLL). Mitotic figures were rare in splenic PDLL and there was no evidence of dissemination. Despite their malignant histology, PDLLs had only minority populations of clonal B cells (Fig. 2; Table 1). The β T-cell receptor locus was not rearranged in these PDLLs. The oligo-polyclonality of these tumours suggests that deregulated *Bcl-2* is the principal contributor to such low-grade neoplasms.

Two lymphomas developed in a comparable number of non-transgenic control littermates over this same period. These differed in that they were intermediate-grade large-cell lymphomas of the spleen without dissemination (Table 1). To estimate the incidence of malignant lymphoma, a cohort of 75 *Bcl-2*-Ig transgenics and 78 control littermates was studied. Autopsies were performed on a total of 21 transgenics and 17 control asymptomatic mice at intervals of 6, 12 and 18 months. Immunoglobulin gene patterns revealed evidence for oligoclonality of B cells by one year of age, but none of these tissues showed any histological evidence of lymphoma. To date, six of the remaining 54 transgenic mice (11%) and one of the 61 control mice (2%) have developed lymphoma in this cohort, which now ranges in age from 12 to 24 months. By comparison, histological conversion to diffuse large-cell architecture occurs in 40–60% of patients with follicular lymphoma but takes 10–16 years³.

The deregulated *Bcl-2* in transgenic lines uniformly leads to an excess of small, resting B cells which accumulate as a result

of extended survival rather than increased proliferation^{12,13}. Because the transgene is present in all cells, this indolent expansion is initially polyclonal. The slow progression to high-grade lymphoma argues for the involvement of secondary genetic changes¹⁶. Synergy between *c-myc* and *Bcl-2* has been demonstrated *in vitro*^{10,17,18}, so we examined the endogenous *myc* genes of these lymphomas. We detected rearrangements in 7 of 15 transgenic DHLs, all of which were immunoblastic (Fig. 3a; Table 1). No *myc* rearrangements were detected in the

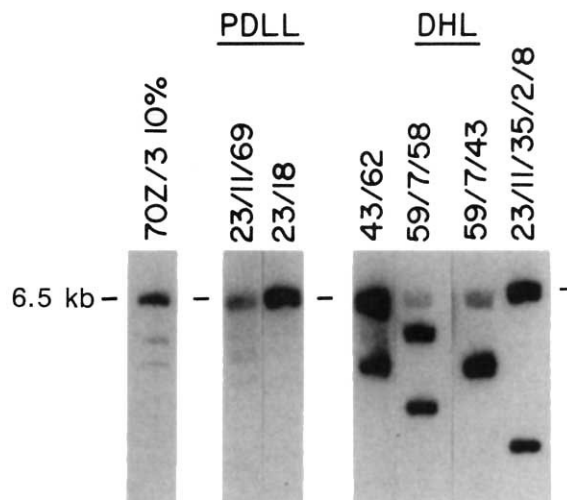


FIG. 2 Southern blot of *Eco*RI-digested DNA from two mice with splenic poorly differentiated lymphocytic lymphoma (PDLL) and from mice with mesenteric diffuse histiocytic lymphoma (DHL). The 70 Z/3 pre-B cell DNA was diluted with liver DNA. The blot was probed with a murine 1.0 kilobase (kb) *Xba*I/*Eco*RI immunoglobulin J_H region probe; the germ-line 6.5-kb *Eco*RI J_H fragment is indicated.

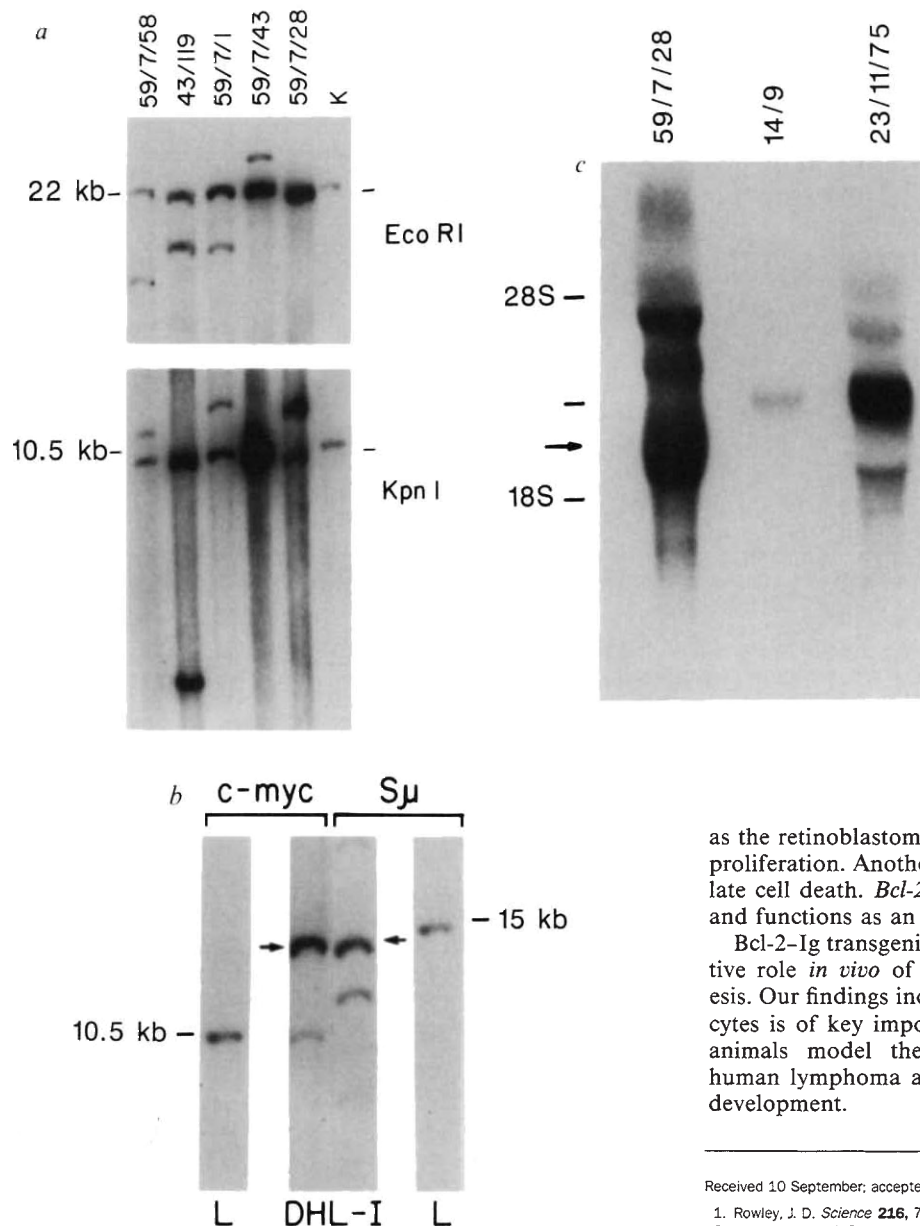


FIG. 3 Analysis of *c-myc* rearrangements in BCL-2-Ig lymphomas. *a*, Southern blots of *Eco*RI- and *Kpn*I-digested tumour DNA from 5 transgenic mice (hybridized with a 3.2-kb *Bam*HI-*Hind*III murine *c-myc* exon II and III probe). Germ-line fragments of 22 kb and 10.5 kb are identified in non-tumorous kidney (K) DNA digested with *Eco*RI and *Kpn*I, respectively. *b*, Southern blot of *Kpn*I-digested tumour (DHL-I) and liver (L) DNA from mouse 59/7/28 probed with the 3.2-kb murine *c-myc* fragment, stripped and rehybridized with a 1.8-kb *Hind*III murine switch μ probe²¹. The 10.5-kb and 15-kb germline *c-myc* and switch μ ($S\mu$) fragments, respectively, are indicated in the lanes of liver DNA. Co-migrating rearrangements are shown (arrows). Similar results were obtained with *Eco*RI-digested DNA. *c*, Northern blot of equal amounts (10 μ g) of total cellular tumour RNA hybridized with the 3.2-kb murine *c-myc* probe. Positions of 18S and 28S ribosomal RNA are indicated. Note the presence of truncated *c-myc* messenger RNA in 59/7/28 tumour RNA (arrow).

as the retinoblastoma and *p53* genes²⁸ inhibit cell growth and proliferation. Another category of proto-oncogenes might regulate cell death. *Bcl-2* would be the first member of this subset and functions as an antidote against death.

Bcl-2-Ig transgenic mice have provided evidence for a causative role *in vivo* of the t(14;18) in malignant lymphomagenesis. Our findings indicate that long-term survival of B lymphocytes is of key importance for further genetic changes. These animals model the tumour progression seen in t(14;18) human lymphoma and offer a means to trace the steps in its development. □

PDLLs or the large-cell lymphomas of non-transgenic littermates. Endogenous *myc* rearrangements in mouse plasmacytomas or Burkitt lymphomas are reciprocal translocations, most frequently involving immunoglobulin heavy-chain switch (S) or joining (J_H) regions¹⁹⁻²². We examined several tumours with *c-myc* rearrangements using probes for immunoglobulin regions J_H , $S\alpha$, $S\gamma 2a$, and $S\mu$ (ref. 23). No J_H -associated translocations were found. A *myc* rearrangement that co-migrated with a $S\mu$ rearrangement in the DHL-I of 59/7/28 is shown in Fig. 3b. In addition, a truncated *myc* messenger RNA was detected (Fig. 3c). These findings are typical of a t(12;15) translocation between the first intron of *myc* and switch regions. These tumours complement their inherent survival advantage with *myc*, which promotes proliferation²⁴. A subset of highly aggressive human lymphoid malignancies also possesses a *myc* translocation, t(8;14), as well as the t(14;18) (refs 25, 26).

The inheritance of deregulated *Bcl-2* can lead to the induction of malignancy, which suggests that *Bcl-2* is a novel type of proto-oncogene. Oncogenes such as *myc*, *ras*, *sis*, *src* and *abl* are growth and proliferation genes involved in cell-cycle progression or signal transduction²⁷; tumour suppressor genes such

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Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1

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THE zinc-finger transcription factor GATA-1 (previously known as GF-1, NF-E1 or Eryf 1 (refs 1-5)) binds to GATA consensus elements in regulatory regions of the α - and β -globin gene clusters²⁻⁶ and other erythroid cell-specific genes⁷⁻⁹. Analysis of the effects of mutations in GATA-binding sites in cell culture and in binding assays *in vitro*^{2,5,10,11}, as well as transactivation studies with GATA-1 expression vectors in heterologous cells¹², have provided indirect evidence that this factor is involved in the activation of globin and other genes during erythroid cell maturation. GATA-1 is also expressed in megakaryocytes^{13,14} and mast cells¹³, but not in other blood cell lineages or in non-haemopoietic cells. To investigate the role of this factor in haematopoiesis *in vivo*,

we disrupted the X-linked GATA-1 gene by homologous recombination in a male (XY) murine embryonic stem cell line and tested the GATA-1-deficient cells for their ability to contribute to different tissues in chimaeric mice. The mutant embryonic stem cells contributed to all non-haemopoietic tissues tested and to a white blood cell fraction, but failed to give rise to mature red blood cells. This demonstrates that GATA-1 is required for the normal differentiation of erythroid cells, and that other GATA-binding proteins^{15,16} cannot compensate for its absence.

To mutate the GATA-1 locus, the positive/negative selection¹⁷ targeting vector pGATA/neo1 (Fig. 1b) was introduced by electroporation into embryonic stem (ES) cells¹⁸. From 6×10^6 cells grown in the presence of the drugs G418 and Gancyclovir, we obtained 79 doubly resistant clones^{17,19}. Counter-selection with Gancyclovir enriches for cells that have lost the herpes simplex virus-thymidine kinase gene through homologous recombination at the target locus¹⁷ (Fig. 1c). Selection of the same number of cells with only G418 yielded 1,800 resistant clones, indicating a potential 23-fold enrichment for homologous recombinants. The 79 clones were individually analysed by Southern hybridization, and six contained the predicted targeted mutation at the GATA-1 locus (Fig. 1d). The apparent ratio of homologous to random integration events was therefore 6/1,800.

Because the GATA-1 gene is X-linked²⁰, the mutant ES cells lacked a normal allele, so we could evaluate the developmental consequences of the mutation in chimaeric mice produced with the mutant cells. Two mutant ES cell clones (53 and 74), each having a euploid XY karyotype, were microinjected into wild-type blastocysts, which were transferred to foster mothers and allowed to develop to term, or to day 14-17 of gestation. Of six live-born chimaeric mice, four (M1-M4) survived to maturity, and two (M5 and M6) failed to thrive as neonates and were sacrificed for analysis (Table 1). We also examined 14 fetal chimaeras. A variety of tissues from the fetal and neonatal chimaeras were analysed to determine the extent of ES cell

TABLE 1 Chimaeras generated with mutant ES cells

148 blastocysts* → 43 pups born						
↓						
6 chimaeric† (4 adults (M1-M4), 2 neonates (M5, M6))						
91 blastocysts* → 48 fetuses <i>in utero</i>						
↓						
14 chimaeric† (9 alive (M7-M15), 5 dead)						
Per cent chimaerism§						
Chimaera‡	Age	ES cell line	Whole blood	Other tissues (range)	Donor Hb	Abnormal phenotype
M1	adult	74	1	15 (10-20)	ND	
M2	adult	74	1	15 (10-20)	NA	
M3	adult	74	ND (<1)	15 (10-20)	NA	
M4	adult	74	ND (<1)	15 (10-20)	NA	
M5	3 days	74	5	40 (30-60)	ND	failed to thrive
M6	7 days	74	NA	20 (10-30)	ND	failed to thrive
M7	17 d.p.c.	53	5	50 (40-60)	ND	anaemic, abnormal liver¶
M8	15 d.p.c.	53	ND (<5)	25 (20-30)	NA	abnormal liver¶
M9	14 d.p.c.	53	5	40 (30-50)	NA	small, pale liver
M10	15 d.p.c.	74	ND (<2)	15 (10-20)	NA	anaemic
M13	14 d.p.c.	74	ND (<2)	25 (10-40)	NA	
M14	14 d.p.c.	74	ND (<2)	40 (30-50)	NA	
M15	14 d.p.c.	74	ND (<5)	35 (30-40)	NA	anaemic

ND, Not detected; NA, not analysed; d.p.c., days post coitum.

* Blastocysts injected with ES cells, transferred to pseudopregnant foster mothers, allowed to develop to term or dissected *in utero*.

† Chimaerism judged by eye pigmentation in fetuses or neonates, and by coat pigmentation in adults.

‡ Chimaeras M11 and M12 did not yield sufficient blood for analysis.

§ Approximate percentage of GPI-1C, estimated by comparison with standard dilutions of GPI-1A and GPI-1C.

|| Values are averages and for chimaeras M1-M4 are based on tail and ear GPI analyses, as well as visual estimation of coat colour chimaerism. Values for other chimaeras are based on five or more tissues.

¶ Liver mottled in colour (probably due to passive congestion, as indicated by histological examination of M7 fetal liver).

contribution, based on a glucose phosphate isomerase (GPI-1) polymorphism between the donor ES cells and the host embryos. All tissues except blood showed extensive ES cell contributions, but the peripheral blood contained very little or no detectable donor-type GPI-1 (Fig. 2a-c; Table 1). In control chimaeras produced with wild-type ES cells, the ES cells contributed to a similar extent to all tissues, including blood (Fig. 2d-f). Therefore the GATA-1 mutation selectively impairs the ability of ES cell derivatives to contribute to the circulating blood cell population.

Because mutant ES cells apparently make a small contribution to the blood in some cases, we next investigated whether the ES-derived blood cells included any mature erythroid cells. Peripheral blood from adult chimaeric mice (M1-M4) was separated into red and white cell fractions. In chimaeras made with the mutant ES cells, only the white cells contained donor-type GPI-1, and none was detected in red cells; but in control chimaeras made with wild-type ES cells, we found equal contributions to red and white cell fractions (see Fig. 2g for example).

As an additional marker to detect any ES-derived mature red blood cells, we examined haemoglobin (Hb) from several chimaeras. The ES cells were homozygous for the *Hbb^d* β -globin haplotype, so that any erythrocytes of ES cell origin would contain Hb major and Hb minor²¹. The recipient MF1-strain embryos carried either the same *Hbb^d* haplotype, or the *Hbb^s* haplotype encoding Hb single. For chimaeras in which the host

embryo was homozygous *Hbb^s*, Hb could be used as a measure of chimaerism; we detected no Hb major or Hb minor in any of these cases (Fig. 2h; Table 1).

These results establish that expression of the gene for GATA-1 is essential for the production of mature, Hb-containing red blood cells, and that the absence of this transcription factor leads to a block in the erythroid cell differentiation pathway. Interestingly, the phenotype of the chimaeric mice indicates that the GATA-1 mutation might interfere with erythropoiesis in a way that does not allow the haemopoietic cells of the host embryo to compensate effectively for the erythropoietic deficit. Of the chimaeric fetuses *in utero*, many were dead or severely anaemic (Table 1). Histological examination of the liver of one such anaemic fetal chimaera (Fig. 3) revealed a fourfold deficiency of erythroid precursors compared with a normal fetal liver; the myeloid cell number was unchanged. Furthermore, chimaeras that survived to adulthood (M1-M4) showed lower ES cell contributions than most of the fetal chimaeras *in utero* (Table 1), suggesting that extensive chimaerism is lethal. In control experiments with wild-type ES cells, there was no mortality or anaemia among fetal chimaeras, several of which developed into highly chimaeric adults (see Fig. 2g, for example). Whether the apparent lethality is due solely to anaemia, or whether the GATA-1 mutation also has detrimental effects on non-erythroid cells, remains to be determined.

Although the exact stage in the erythroid lineage at which the

FIG. 1 Targeted disruption of the GATA-1 gene. *a*, Wild-type GATA-1 gene, indicating exons IA through to VI, based on complete DNA sequence (S.-F.T. and S.H.O., unpublished). Restriction enzyme sites: B, *Bgl*II; P, *Pvu*II; S, *Sac*I; N, *Nco*I; E, *Eco*RI; D, *Dra*I. *b*, Targeting vector pGATA/neo1, including a *neo* gene inserted immediately upstream of the initiation codon in exon II, and the herpes simplex virus thymidine kinase (HSV-tk) gene. *c*, Structure of the mutated GATA-1 gene after homologous recombination. *d*, Southern analysis of wild-type CCE (ref. 17) ES cells (lanes C) and three cell lines (5, 33 and 44) in which the GATA-1 gene was disrupted by homologous recombination. The probe is an *Eco*RI-*Dra*I fragment indicated in *a* and *c* (solid bar). Migration positions of size markers (kilobases, kb) are indicated to the left. The sizes of the bands in wild-type and mutant DNAs, respectively, are: *Bgl*II, 4.5 and 5.6 kb; *Eco*RI, 2.9 and 2.0 kb; *Pvu*II, 5.9 and 4.0 kb; *Sac*I, 3.8 and 4.9 kb. Similar results were obtained (not shown) with the other three homologous recombinant cell lines.

METHODS. Plasmid pGATA/neo1 was constructed using a 4.8-kb *Pst*I partial restriction fragment, including exons IB-V and part of exon VI (Fig. 1b), cloned in PUC19. A unique *Nco*I site in exon II was converted to a *Xho*I site, and the 1.1-kb *Xho*I-*Sal*I fragment from pMC1neoPA (a derivative of the pMC1neo gene²⁷, which includes a poly(A) addition site; Stratagene) was inserted there. This plasmid was digested with *Hind*III and *Sal*I (in the PUC19 polylinker) and a fragment containing GATA-1 sequences and the pMC1neoPA gene was transferred to pBluescript II Ks+ containing HSV-tk. Although the ATG initiation codon of the GATA-1 gene remained intact in pGATA/neo, any expression of GATA-1 protein by the mutant allele is likely to be minimal, owing to efficient cleavage and polyadenylation directed by the synthetic poly(A) site in the pMC1neoPA cassette, and to the low efficiency of translational reinitiation on a bicistronic messenger RNA. Indeed, no GATA-1 protein could be detected in extracts of the mutant ES cells by a band-shift assay (not shown), although the pMC1neoPA gene was active in these cells. pGATA/neo1 DNA was digested with *Xba*I and *Sal*I (which cleave in the polylinker, liberating from the plasmid vector the fragment shown in *b*), and 25 μ g digested DNA was used to electroporate 2×10^7 CCE ES cells¹⁹. Cells were plated and subjected to G418/Gancyclovir selection as described^{17,19,28}; surviving colonies were isolated and grown into cell lines.

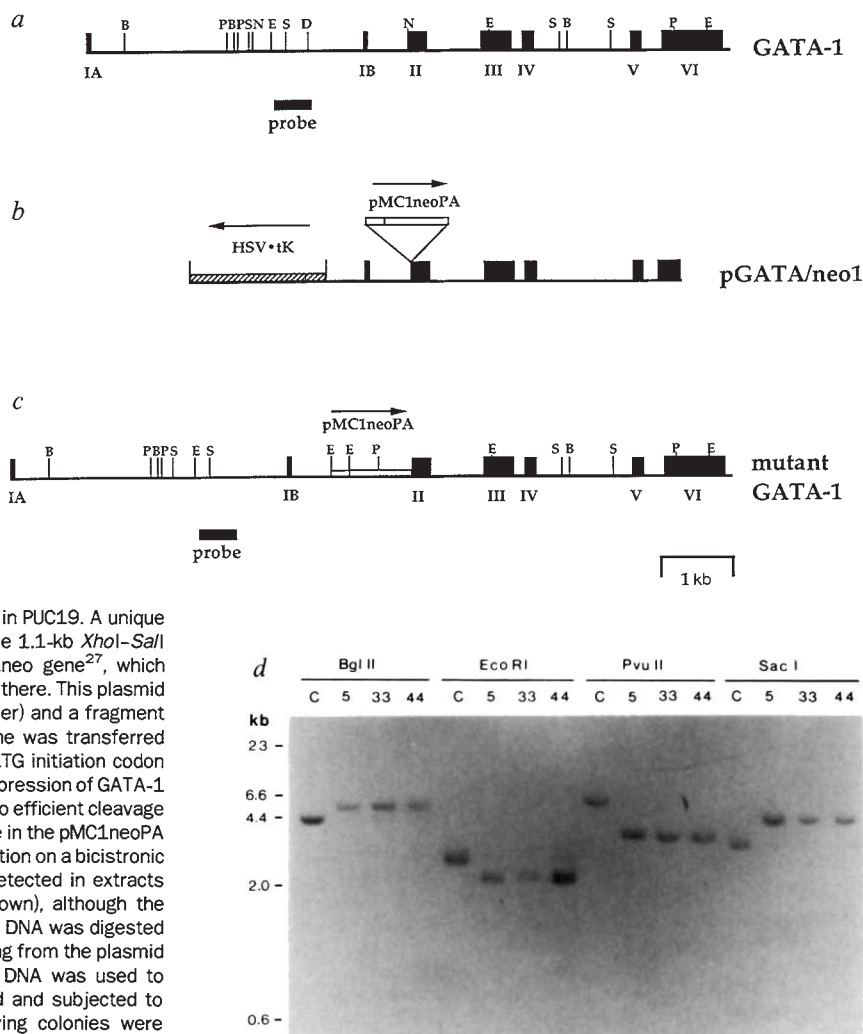
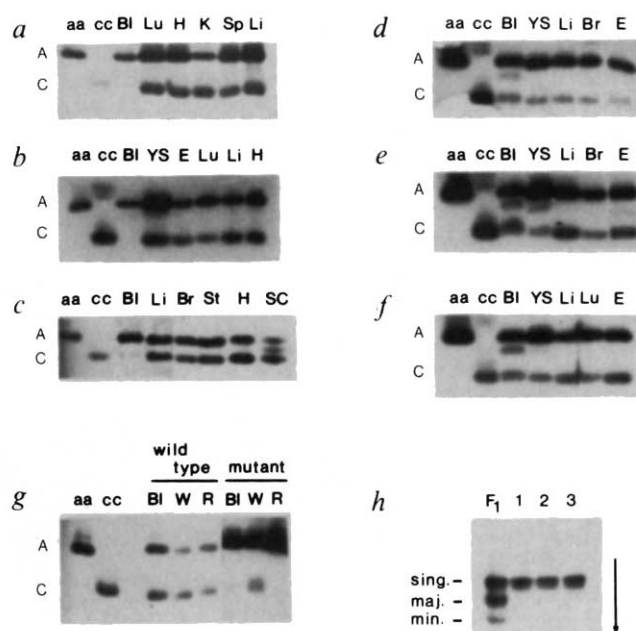


FIG. 2 Contribution of GATA-1-deficient or wild-type ES cells to tissues in chimaeric mice. The relative proportion of glucose phosphate isomerase (GPI-1) isozymes A and C was assayed in tissue extracts from chimaeric mice killed at the fetal or postnatal stage. Cells derived from the host blastocysts contain the *Gpi-1s^a* allele (encoding the GPI-1A isozyme); cells derived from the injected ES cells contain the *Gpi-1s^c* allele (encoding GPI-1C). *a-c*, Results for 3 chimaeras (M5, M8 and M7, respectively) produced with mutant ES cells. *d-f*, Results from 3 chimaeras produced with the parental, wild-type ES cells, and sacrificed at 15, 16 and 16 days of gestation, respectively. Lanes labelled aa and cc contain control blood samples from mice homozygous for *Gpi-1s^a* or *Gpi-1s^c*; Bl, whole blood; Lu, lung; H, heart; K, kidney; Sp, spleen; Li, liver; YS, yolk sac; E, eye; Br, brain; St, stomach; SC, spinal column. The intermediate band in *c*, lane SC, is a GPI-1A/GPI-1C heterodimer formed in multinucleate muscle cells. The intermediate band in some control blood and yolk sac samples (*d-f*) is the GPI-1B isozyme derived from contaminating maternal blood (the foster mothers were homozygous for the *Gpi-1s^a* allele). *g*, Results from whole blood (Bl), total white blood cells (W) and total red blood cells (R) from two adult chimaeric mice, one produced with wild-type ES cells and one with mutant ES cells (chimaera M1). *h*, Analysis of haemoglobins in whole blood of three chimaeric mice produced with mutant ES cells. Lane F₁, blood from a normal (CBA/JxC57BL/6J)F₁ mouse, heterozygous for the *Hbb^d* and *Hbb^s* haplotypes at the β -globin locus. Lanes 1, 2 and 3, blood from chimaeras M5, M7 and M6, respectively. All three chimaeras contain only Hb single (sing.), produced by host embryo-derived red cells, and no detectable Hb major (maj.) or Hb minor (min.), which would be produced by any ES cell-derived red cells. Arrow denotes direction of electrophoresis.

METHODS. ES cells were microinjected into strain-MF1 blastocysts, which were implanted into pseudopregnant foster mothers as described²⁸. Tissues were dissected from the resulting chimaeric fetuses or mice, and analysed for GPI as described²⁹. Red and white blood cell fractions were obtained by



centrifugation of heparinized whole blood in microcrit capillaries. Haemoglobins were analysed by cellulose acetate electrophoresis of haemolysates treated with cystamine³⁰.

GATA-1 mutation exerts its effects has not yet been identified, our observations may be pertinent. If the mutation were deleterious to the pluripotent haemopoietic stem cells (HSC), the normal host HSC pool in a chimaeric fetus would be expected to proliferate and replenish the erythroid compartment. For example, a small number of normal HSC introduced into anaemic W/W fetuses, which suffer from an HSC deficiency²², can reconstitute the haemopoietic system and correct the anaemia²³. The apparent lack of such compensation indicates that the GATA-1 mutation may not reduce the number or proliferative capacity of the HSC. The viability of GATA-1-deficient HSC is also demonstrated by the ability of the mutant ES cells to contribute to at least a subset of white blood cells (Fig. 2g). Instead, the mutation most probably interferes at a

later stage in progenitor development leading to the erythroid lineage.

Although earlier studies strongly suggested that GATA-1 has a role in erythroid cell-specific gene expression, we have now obtained direct evidence for such a role by analysing the effects of a specific mutation on erythropoiesis *in vivo*. This finding is particularly significant in view of the discovery of other transcription factors with zinc-finger DNA-binding domains very similar to that in GATA-1, in chicken erythrocytes¹⁵ and in mammalian cells¹⁶. As these proteins also bind to GATA motifs and can activate transcription in heterologous cells, the possibility of functional redundancy between multiple regulatory factors in erythroid cells must be considered. Our results, however, demonstrate that other GATA-binding proteins cannot com-

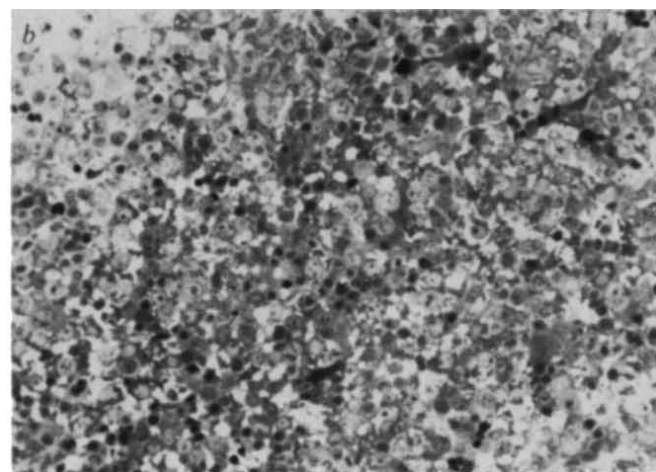
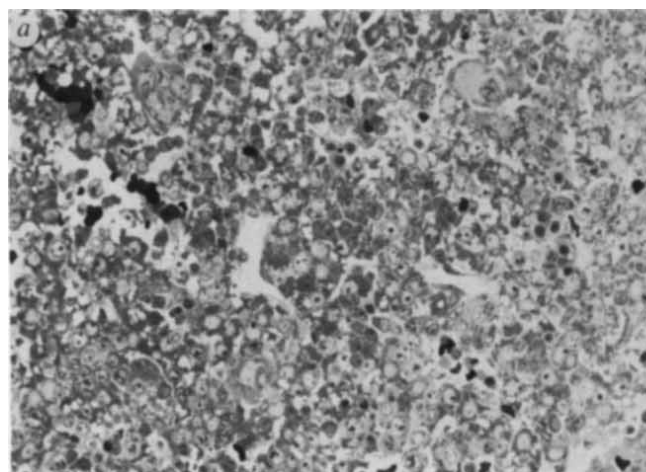


FIG. 3 Deficiency of erythroid cells in the liver of a 17-day chimaeric fetus produced with mutant ES cells. *a*, Histology of the liver from chimaera M7, derived ~50% from mutant ES cells and 50% from normal host cells, based on GPI analysis (Fig. 2c). *b*, Histology of a normal fetal liver from a non-chimaeric littermate. Erythroid precursors, recognizable by their darkly stain-

ing nuclei, are widespread in the normal fetal liver but relatively scarce in the chimaeric liver.

METHODS. Fragments of fetal liver were fixed in formalin, post-fixed in osmium tetroxide, and embedded in Epon. Sections were cut at one micron and stained with toluidine blue.

pensate for a deficiency of GATA-1 during erythroid development. Gene targeting should also be useful in delineating the individual developmental functions of other transcription factors with potential redundancy, such as the multiple octamer-binding proteins²⁴.

Although most autosomal recessive mutations produced in ES cells must be bred to homozygosity before any phenotypic effects are evident^{25,26}, the fact that male cells are hemizygous for the X-linked GATA-1 gene has enabled us to examine the consequences of a targeted mutation through the direct analysis

of chimaeric mice. If mutant ES cells can contribute to the germ line in viable chimaeric males, it will be interesting to follow the development of embryos with cells uniformly deficient in GATA-1. Meanwhile we can use chimaeras to provide an answer to questions such as whether this factor is also necessary for the development of primitive, yolk sac-derived erythroid cells, or for the differentiation of megakaryocytes or mast cells. Finally, the mutant ES cells provide a null background in which altered versions of the GATA-1 gene can be introduced to test the effects of directed mutations on erythroid development.

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Ultrastructural basis and function of iridescent blue colour of fruits in *Elaeocarpus*

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IRIDESCENT colour, caused by physical effects (thin-film interference, diffraction and Tyndall scattering), is relatively common in animals but exceedingly rare among plants¹. Some benthic marine algae produce blue to violet iridescence^{2,3}, and the upper leaf surfaces of a few vascular plants from the shady environments of humid tropical forests are iridescent blue^{4–6}. Blue fruit colour has been assumed to be caused by anthocyanins⁷. A survey of such fruits (26 species in 18 genera) in Costa Rica, India, Florida and Malaysia, showed this to be the case, except for the iridescent colour in fruits of *Elaeocarpus angustifolius* Blume (Elaeocarpaceae). There I show that the colour is caused by a remarkable structure in the epidermis, and provide evidence for its selective advantage.

The fruits of most of the 60 species of *Elaeocarpus*, a genus of trees native to the Asian tropics and Australasia, are blue⁸. Those of *E. angustifolius* (syn. *E. sphaericus* and *E. grandis*) produce a particularly brilliant colour, but no pigments can be extracted with acidic methanol. E. J. H. Corner wrote⁹ that “the blue colour of the cuticle is caused, not by a blue pigment, but by the structure of the cuticle which reflects blue light”. The colour is clearly limited to the epidermis of the fruit and is not removed by contact with water, indicating that the structural basis is in the cell layer^{10,11}. Diffuse reflectance measurements of the fruit surface (Fig. 1) peak at 439 ± 2 nm (s.e.m.; $n = 5$). Because the colour is uniform at different angles, and reflectance does not increase at shorter wavelengths, both diffraction and Tyndall scattering can be excluded as the basis of colour production¹. Assuming a refractive index of 1.35–1.45 for the hypothetical structure¹², the thickness of layers responsible for

colour production can be predicted using the standard formula for thin-film interference¹³: thickness = $\lambda / 4\mu \cos \theta$ where θ is refracted angle of light in the filter, μ the refractive index of the film, and λ the peak wavelength for constructive interference. This equation predicts a thickness of 76–81 nm, assuming the refractive index of the filter is greater than the surrounding medium (otherwise the thickness would be twice that predicted).

Light (Fig. 2a) and transmission electron microscopy (Fig. 2b–d) reveal a remarkable structure beneath the outer cell walls of the adaxial epidermis. This structure, termed an iridosome (based on analogous structures in animals¹), consists of a roughly parallel network of electron-translucent strands each 78 ± 4 nm thick ($n = 5$; each value is the mean of 10 measurements within an iridosome of each fruit). Strands are separated by lacunae of 39 ± 5 nm, and paradermal sections of the iridosome (Fig. 2d) reveal straight rows (62 ± 3 nm wide and $62 \pm$

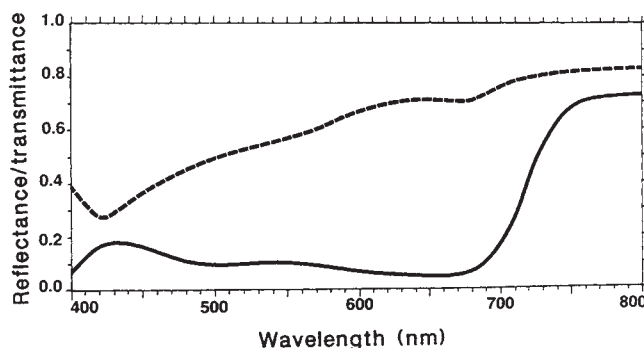
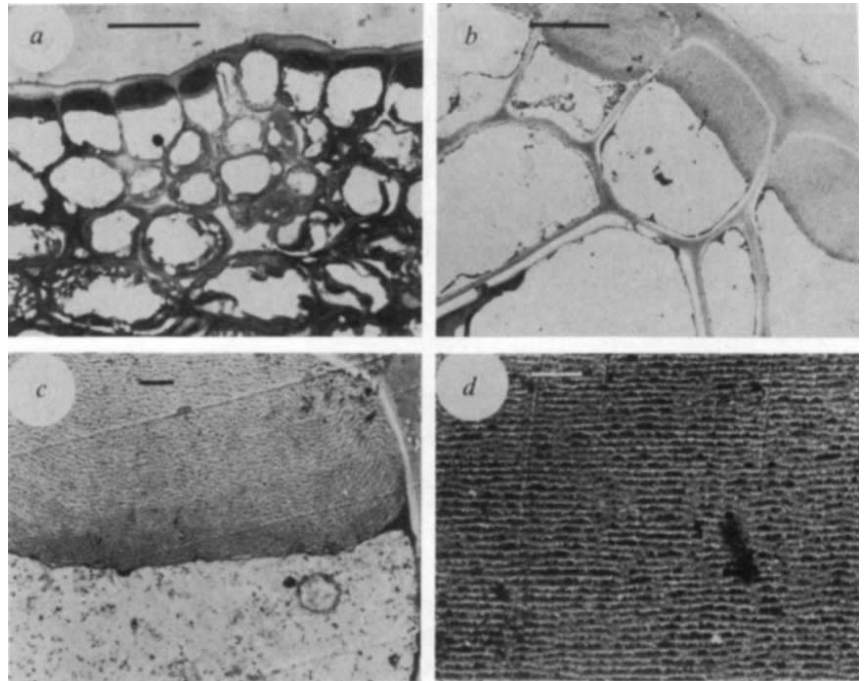


FIG. 1 Optical properties of the iridescent blue fruits. Fruits were collected from two trees at the USDA National Clonal Germ Plasm Collection in Miami. Diffuse reflectance of the intact fruit wall (compared to an optically white barium sulphate surface) was measured with a Li-Cor 1800 spectroradiometer attached by a fibre optic probe to an integrating sphere (Li-Cor Instruments, Lincoln, Nebraska 68504). Transmission through the epidermis was measured by the spectroradiometer attached via a fibre-optic probe to the photographic tube of a Leitz dialux microscope using the $\times 40$ objective (transmission in the absence of tissue was divided into the scan). Dotted line, transmittance; solid line, reflectance.

FIG. 2 Micrographs of fruit tissue, showing the location and structure of the iridosome. *a*, Light micrograph of tissue (1- μ m thick, stained with toluidine blue). Iridosomes are located immediately beneath the epidermal cell walls. Bar, 20 μ m. *b-d*, Transmission electron microscopy photographs: *b*, transverse section, layers within the iridosome are clearly seen. Bar, 10 μ m. *c*, Detail of the strands in the iridosome. Bar, 1 μ m. *d*, Paradermal section of fruit epidermis, showing contiguity of each band. Bar, 1 μ m.

METHODS. Fruit tissue was fixed in 3% glutaraldehyde and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer at pH 7.2. The tissue was dehydrated in an acetone series, embedded in Spurr's low-viscosity resin, and thin sections were cut in a Porter Blum ultramicrotome for microscopy. Grids were stained with lead citrate and uranyl acetate and photographed in a Philips 200 transmission electron microscope. Structures were measured from original negatives using a dissecting microscope and stage micrometer.



3 nm apart). The two views suggest that the iridosome consists of strands organized as a three-dimensional lattice. This structure should intensify colour production, similar to the wing scales of morpho butterflies (*Morpho* sp.)^{1,14,15} and the feather barbles of the peacock (*Pavo cristatus* L.)^{1,16}.

The iridosome is not present in the epidermal cells of immature green fruits. In mature fruits it is extracellular, lying between the adaxial wall and the cytoplasmic membrane. The iridosome seems to be secreted by the cytoplasm; its thickness increases with the intensification of blue coloration in the maturing fruits, and the layers next to the cytoplasm are more electron-dense and more closely appressed (Fig. 2, *b, c*).

Histochemical staining reveals that the layers of the iridosome consist of polysaccharides, at least partly of cellulose. Iridosomes stain positively for polysaccharides with periodic acid Schiff's reagent¹⁷ and for cellulose with Calcofluor White M2R (ref. 18), and negatively for lignins by the acid phloroglucinol test and for callose by the aniline-blue fluorescence test¹⁹. The structure is strongly birefringent under polarized light microscopy, suggesting that the polysaccharides are present as linear fibrils.

Iridescent blue may be of selective advantage in the ripe and senescent fruits of *E. angustifolius* in two ways. Although red and black seem to be stronger attractants, 5–7% of the fleshy fruits consumed and dispersed by birds are blue²⁰. The fruits of *E. angustifolius* contain a fleshy mesocarp (rich in carbohydrate and protein; E. D. Stiles, unpublished results) surrounding an extremely hard endocarp. They are important in the diets of the cassowary (*Casuaris casuaris*), fruit-eating pigeons and small mammals^{21–24}. Not only do the iridescent fruits present a brilliant colour against the green foliage or brown litter on the forest floor, but the colour persists even when the mesocarp is almost completely senescent or has been consumed by beetles.

Photosynthesis in unripe or green ripe fruits contributes greatly to the carbon economy of many plants^{25,26}. In fruits producing colour through pigmentation, however, most of the visible wavelengths (400–700 nm) are absorbed by the epidermis except those producing the colour, which are scattered in internal tissues and diffusely reflected²⁷. The most brilliantly coloured fruits are those that scatter light most efficiently, such as of *Heliconia*, which have a white unpigmented mesocarp below the blue epidermis. The epidermis (or exocarp) of *E. angustifolius* fruits is transparent at higher wavelengths, where destructive

interference occurs (Fig. 1), and should allow the penetration of photosynthetically active radiation above 500 nm to reach the chlorenchymatous tissue in the ripened fruits. The mesocarp of ripened fruits of *E. angustifolius* is relatively rich in chlorophyll ($136 \pm 37 \mu\text{g cm}^{-2}$, $n=5$; measured according to Arnott²⁸), more than the leaves on an area basis but less per unit volume given the 4-mm thickness of this layer^{29,30}.

I tested the photosynthetic contribution to the carbon economy by measuring carbon dioxide emissions of fruits under field conditions, at 26 °C and $160\text{--}175 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400–700 nm (photosynthetically active radiation, PAR) in diffuse canopy shade of ~8% full sunlight. Fruit-temperature, CO_2 production and PAR were measured beneath a tree crown at Fairchild Tropical Garden, Miami, with a Li-Cor 6000 photosynthesis system. Five ripened fruits were measured simultaneously in a 0.25-l chamber, with 10 repetitions of different combinations of 20 fruits, on 2 days. Fruits were left in darkness for 60 min, their CO_2 emissions measured in the dark, transferred to diffuse shade-light for 60 min, then measured again. Diminished CO_2 production was detected under these low light conditions. Higher PAR levels could not be used because increased fruit temperatures could increase respiration. Fruits exposed to light emitted $9.2 \pm 0.2\%$ less CO_2 than those kept in the dark, which respired at $0.28 \pm 0.03 \text{ mg m}^{-2} \text{s}^{-1}$ of CO_2 . Fruits were capable of exchanging gases through stomata and lesions developing from the detachment of trichomes. Thus, photosynthesis by ripened and senescing fruits of *E. angustifolius* contributes to the carbon economy of the plant even under shady conditions. □

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A Cu(I)-semiquinone state in substrate-reduced amine oxidases

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THE role of copper in copper-containing amine oxidases has long been a source of debate and uncertainty¹. Numerous electron paramagnetic resonance (EPR) experiments^{2–6}, including rapid freeze-quench studies⁷, have failed to detect changes in the copper oxidation state in the presence of substrate amines. One suggestion that copper reduction might occur⁸, has never been confirmed. Copper amine oxidases contain another cofactor, recently identified as 6-hydroxydopa quinone (topa quinone)⁹, which is reduced by substrates. Copper has been implicated in the reoxidation of the substrate-reduced enzyme^{10–12}, but the failure to detect any copper redox change has led to proposals that Cu(II) acts as a Lewis acid¹³, that it has an indirect role in catalysis¹⁴, or that it serves a structural role⁶. We present evidence for the generation of a Cu(I)-semiquinone state by substrate reduction of several amine oxidases under anaerobic conditions, and suggest that the Cu(I)-semiquinone may be the catalytic intermediate that reacts directly with oxygen.

EPR spectral changes accompanying the addition of an appropriate amine to several amine oxidases under anaerobic conditions are shown in Fig. 1. In each case, a relatively sharp signal at $g \sim 2$ is observed, although the yield of this species is variable. Scans of the $g \sim 2$ signal at higher resolution (Fig. 2) show that the same radical is formed in different amine oxidases. Because the radical EPR spectrum is independent of the enzyme and substrate used, the radical must be associated with a moiety that is conserved among the amine oxidases examined. The spectra in Fig. 2a are essentially identical to the EPR spectra of amine oxidases that had been anaerobically reduced in the presence of cyanide or *t*-butylisocyanide (refs 5, 10, 15); these ligands are thought to trap the semiquinone form by stabilizing Cu(I). Addition of cyanide to reduced porcine plasma amine oxidase dramatically increases the intensity of the weak $g \sim 2$ signal (evident in Fig. 1), thereby allowing the characteristic hyperfine structure to be resolved (Fig. 2b). The assignment of the $g \sim 2$ signal to a semiquinone radical is supported by a variety of evidence^{10,15}, including pulsed-EPR studies (J. McCracken, J. Peisach and D.M.D., manuscript in preparation).

Double integration indicates that the yield of the semiquinone in the absence of cyanide ranges from <1% (porcine plasma amine oxidase) to ~20% (pea seedling diamine oxidase and *Arthrobacter* P1 methylamine oxidase) of the theoretical maximum, calculated from the enzyme concentration and assuming a 1:2 protein:quinone stoichiometry^{10,16}.

Two independent methods were used to quantitate the Cu(II) EPR signal in the substrate-reduced forms of pea seedling diamine oxidase and *Arthrobacter* methylamine oxidase. Vännegård has shown that the area of the $M_I = -3/2$ hyperfine line in a first-derivative Cu(II) EPR spectrum is proportional to the total intensity¹⁷; comparison of these peak areas for the resting and substrate-reduced enzymes, obtained under identical conditions, indicates that ~40% of the Cu(II) is reduced. Alternatively, the contribution of the sharp $g \sim 2$ signal was mathematically subtracted from the EPR absorption envelope, which was then integrated to give the Cu(II) intensity. Again using the resting enzyme as a standard, the results were in good agreement with those obtained from the integration of the lowest hyperfine line. A lower yield of the semiquinone, relative to the

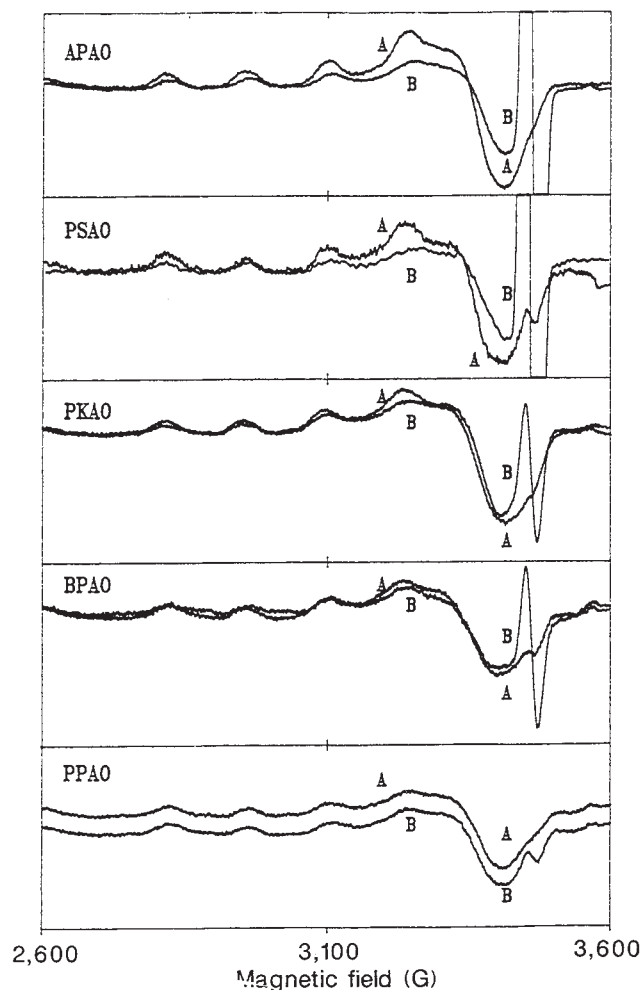


FIG. 1 Room-temperature EPR spectra of amine oxidases from *Arthrobacter* P1 (APAO), pea seedling (PSAO), porcine kidney (PKAO), bovine plasma (BPAO) and porcine plasma (PPAO), before (A) and after (B) anaerobic addition of substrate. Spectra were obtained in 0.3 mm × 6 mm × 100 mm glass microslides (Vitro Dynamics) on a Bruker 220D SRC instrument immediately after samples were prepared and sealed in a nitrogen atmosphere glove box. Frequency, 9.8 GHz; power, 20 mW; modulation amplitude, 20 G; [enzyme], 0.3–0.8 mM in 50 mM barbital, pH 8.0 (APAO, BPAO, PPAO), 50 mM PIPES, pH 7.0 (PSAO), or 0.1 M phosphate, pH 7.2 (PKAO); [substrate], 1–3 mM benzylamine (APAO, BPAO, PPAO) or cadaverine (PSAO, PKAO). The PPAO spectra are offset for clarity.

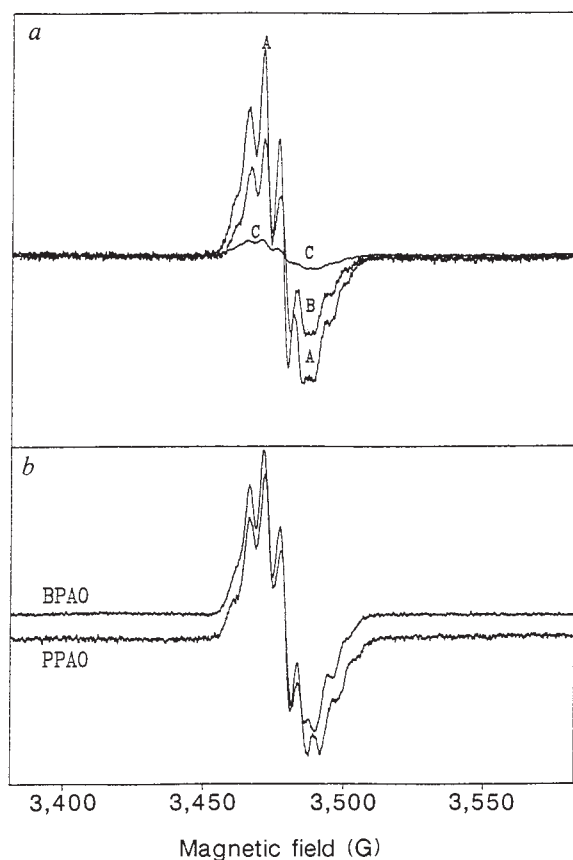


FIG. 2 EPR spectra showing radical formation in amine oxidases after anaerobic addition of substrate. See Fig. 1 legend for particulars. *a*, Spectrum obtained in the absence of cyanide. A, APAO; B, PSAO; C, PKAO. *b*, Cyanide (5 mM) was used to enhance the radical signal in BPAO and PPAO, whose spectra are offset for clarity. Frequency, 9.8 GHz; power, 6.3 mW (40 mW for PKAO); modulation amplitude, 1 G; room temperature.

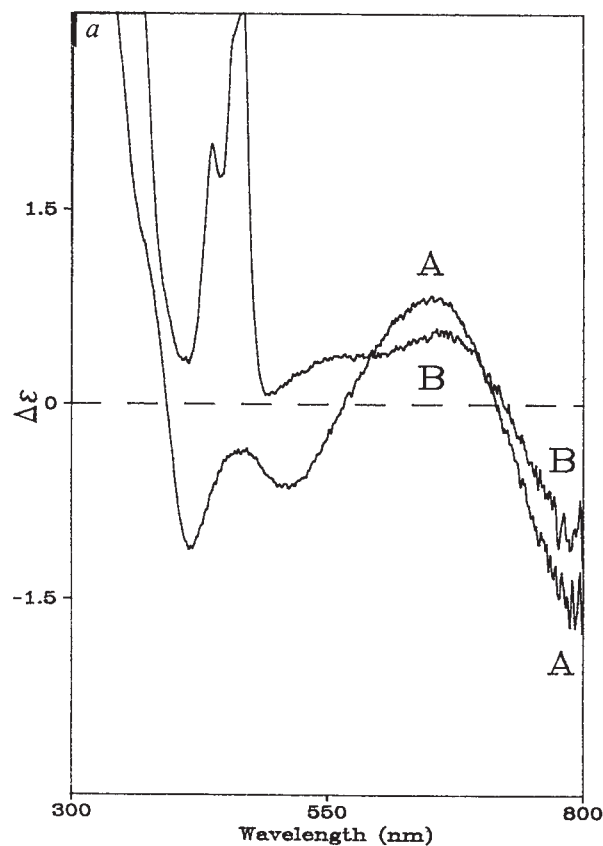


FIG. 4 *a*, CD spectra ($\Delta\epsilon = \epsilon_L - \epsilon_R$, $M^{-1} cm^{-1}$) of native APAO (A) and of APAO after anaerobic addition of a four-fold excess of benzylamine (B), in 0.1 M phosphate, pH 7.2, 295 K. *b*, Visible absorbance spectra of APAO, showing the effect of temperature on the radical (top) and on the cyanide-

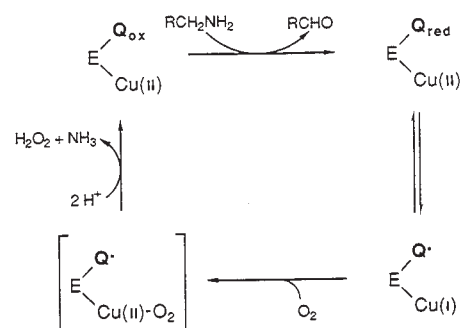
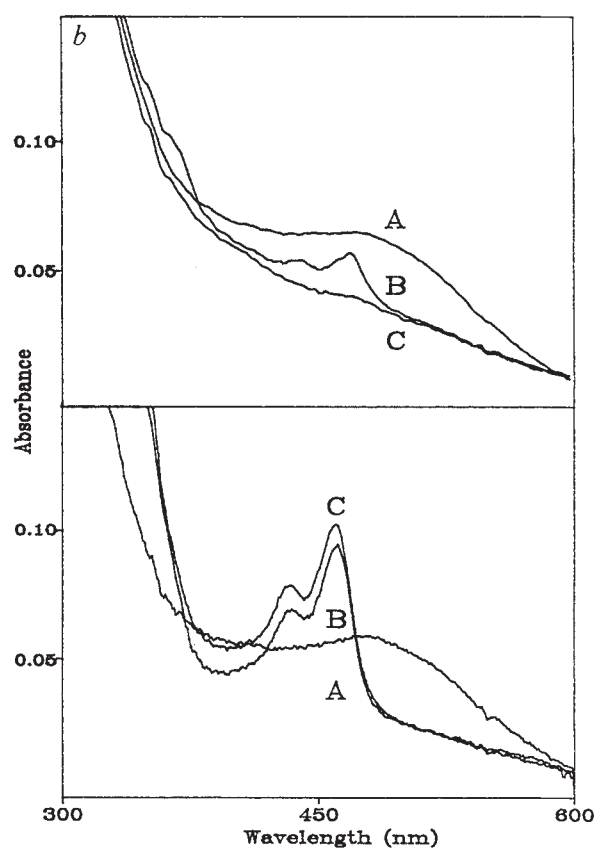


FIG. 3 Possible catalytic cycle of copper-containing amine oxidases. The species in brackets is a hypothetical intermediate, shown here to emphasize the possibility of sequential one-electron steps in the reduction of O_2 . Q_{ox} , oxidized quinone; $Q^\bullet$, semiquinone; Q_{red} , two-electron reduced quinone.



stabilized radical (bottom). (A) Native APAO (15 μM), 10 mM phosphate in methanol/water (1/4), pH 7.5, 295 K; (B) APAO + 60 μM benzylamine, 295 K; (C) APAO + benzylamine, 258 K. Pathlength, 1 cm; [NaCN], 5 mM.

decrease in the Cu(II) EPR signal, can be attributed to inter- or intramolecular disproportionation, that is $2 \text{ Cu(I)}\text{-Q} \rightleftharpoons \text{Cu(I)}\text{-Q}_{\text{red}} + \text{Cu(I)}\text{-Q}_{\text{ox}}$, using the notation of Fig. 3. Copper reduction by substrates was confirmed by circular dichroism (CD) spectroscopy. The Cu(II) ligand-field transitions of pea seedling diamine oxidase and *Arthrobacter* methylamine oxidase are observed in the CD spectra from 600 to 800 nm (ref. 10, and D.M.D., unpublished observations), where the quinone makes no contribution. Fully reduced amine oxidases display no CD bands in this region. Substrate addition under anaerobic conditions produces a 35% decrease in the intensity of the methylamine oxidase Cu(II) CD bands (Fig. 4a), consistent with the EPR results.

The EPR data in Figs 1 and 2 establish that amine oxidase Cu(I)-semiquinones can be generated under conditions that are biologically relevant, in the absence of trapping agents. This permits the development of a plausible mechanism (Fig. 3) featuring well-precedented roles for both copper and the quinone, which circumvents the well-known spin conservation problem associated with two-electron reductions of oxygen¹⁸. Evidence for a bound superoxide intermediate has been previously described^{19,20}. Further, for an amine oxidase isolated from lentil seedlings, Finazzi-Agro and co-workers have shown that a substrate-reduced species with absorption bands at 464, 432 and 360 nm (similar to B in Fig. 4b) is a catalytic intermediate and reacts very rapidly with oxygen²¹. By correlating the room-temperature EPR and absorption spectra, this intermediate can plausibly be assigned as the Cu(I)-semiquinone.

Why has the Cu(I)-semiquinone state of amine oxidases been so elusive? The results in Fig. 4b provide a clue. Anaerobic addition of substrate to methylamine oxidase produces the characteristic semiquinone absorption bands; cooling, without freezing, the enzyme solution bleaches the absorption spectrum and produces a spectrum that represents a mixture of the fully reduced and oxidized quinones. The process is reversible because the semiquinone bands are recovered upon warming the solution to 22 °C. Cyanide prevents the bleaching of the semiquinone spectrum on cooling, probably by stabilizing Cu(I) and preventing back electron-transfer, thereby allowing the semiquinone to be observed even at low temperature. Collectively, the results suggest that the Cu(I)-semiquinone and the Cu(II)-reduced quinone are in equilibrium, and that low temperatures favour the Cu(II) form. Similar effects might be present in other enzymes containing multiple redox centres where intramolecular electron-transfer steps are involved in the mechanism. □

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ERRATUM

Anticrack-associated faulting at very high pressure in natural olivine

Harry W. Green II, Thomas E. Young, David Walker & Christopher H. Scholz

Nature **348**, 720–722 (1990)

IN this letter in the 20/27 December issue, the legend to Fig. 1 should read:

FIG. 1 Low-magnification photomicrograph in obliquely incident light showing a fault traversing specimen 36. The specimen was shortened in approximately the E–W direction. Bright areas on the edges of the specimen are remnants of the rhenium heater. Vertical dimension of specimen is 3 mm.

The printed version referred incorrectly to the 'N–S direction' and the horizontal dimension.

CORRECTION

Weak-link-free behaviour of high-angle YBa₂Cu₃O_{7-δ} grain boundaries in high magnetic fields

S. E. Babcock, X. Y. Cai, D. L. Kaiser & D. C. Larbalestier

Nature **347**, 167–169 (1990)

THE expression used to calculate J_c values throughout the above paper was misquoted in the paper (bottom of left side, page 332) as $J_c = \Delta M / (2r)$. The correct expression is $J_c = 3\Delta M / r$. The correct expression was used for all the calculations which appear in the text and figures.

Go with the flow

A supernatant removal system, robotic liquid handlers to automate repetitive tasks in the laboratory and a software-assisted concentrator/filtrator — tools to ease liquid handling tasks.

THE **microcentrifuge tube caddy** from Research Products International is designed to shield the user during the handling and transportation of radioactive samples (*Reader Service No. 101*). As the tube caddy is made of 0.375-inch thick acrylic, radiation exposure from beta emitters such as ^{32}P is reduced, says RPI. The \$19 (US) caddy, which can hold up to four 0.5-ml and up to eight 1.5/2-ml microcentrifuge tubes, features a hinged lid and non-skid rubber feet.

High production capacity is a feature of the Bellco **Hi-Pro roller apparatus**, an 86-bottle position system that is available from Bibby Sterilin (*Reader Service No. 102*). The Hi-Pro apparatus provides up to



Hi-Pro high-capacity roller apparatus.

136,000 cm² of surface area and is suitable for rolling both glass and plastic disposable roller bottles. Bottles can be set to rotate at speeds of between 0 and 3 rpm ± 0.03 rpm, says Bibby. The apparatus is available in two heights: 72 cm and 84 cm. The easy-out bottle guide facilitates safe loading.

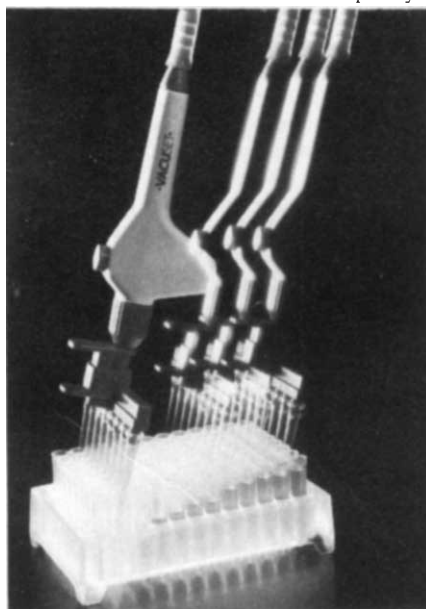
For high-quality purified water 'on tap', Elga recommends its Elgastat Option 3 and 4 models, which are compact bench or wall-mounted **water purification units** (*Reader Service No. 103*). The Option 3 model, a single-pass unit that feeds directly into a storage vessel, combines a composite pretreatment cartridge with reverse osmosis, an ionic/organic removal cartridge and UV disinfection to produce up to eight litres of purified water per hour. According to Elga, the new units can produce significant savings in running costs: for example, producing eight litres of purified water using Option 3 consumes 56 W of energy as compared to the 6,000 W that would be consumed when producing the same amount and quality of water in a typical cabinet still. Option 4 has all the features of the Option 3 model but, in addition, incorporates an internal

pump for the continuous recirculation of purified water.

Liquid dispensing/removal

Jencons Scientific has introduced a general purpose **liquid dispenser**, called the Perimatic GP, which can operate in either manual or automatic modes (*Reader Service No. 104*). The dispenser offers two pumping speeds, a reverse pumping facility, and slow-start, slow-finish options that are designed to prevent frothing and splashing, particularly when filling Petri dishes and small containers. A built-in microprocessor controls the dispensed volume and a LED indicates which functions are in use.

The latest gadget from Inotech is the Vacuset **supernatant removal system** for the collection and disposal of waste fluids from tissue culture, blood samples, washed cells or sediments (*Reader Service No. 105*). The handpiece accepts a variety of adapters, including an eight-channel pipettor with ejector for disposable tips. This set up is intended for use with 96-well microtitre plates and for removing sera and washes from western blot strip trays.

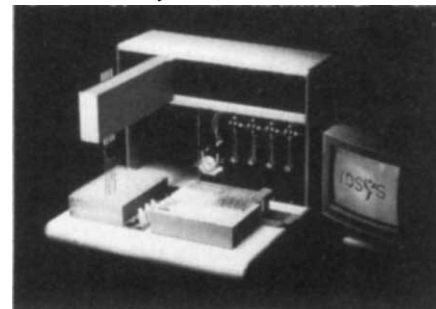


Inotech's complete fluid removal system.

The complete system — handpiece, adapters, tubing and collection bottles — is autoclavable and costs \$284 (US). The safety-coated collection bottles have a three-litre capacity and feature double-sealing quick connectors that are designed to prevent drip loss. A vacuum controller is an optional extra.

Lab automation

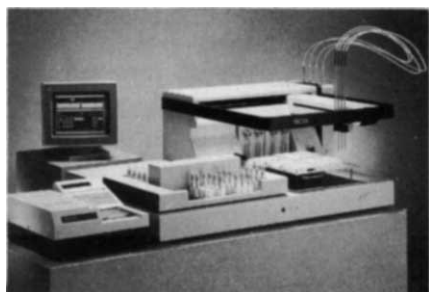
A new generation of multi-tipped **robotic liquid handlers** that are designed to automate any laboratory pipetting procedure — EIAs, ELISAs, FIAs and RIAs — is available from the Swiss-based company, Rosys (*Reader Service No. 106*). Two models are available: the alpha robotic liquid handler with four Teflon-coated stainless steel tips, each of which can be washed thoroughly both inside and out to minimise carryover; and the beta model



Rosys' multi-tipped robotic liquid handlers with washable or disposable tips.

with disposable tips for applications where zero carryover is essential (for example, sterile work). The four tips in both models have liquid-level detection capabilities. In addition, the spacing between the tips can be varied so that any combination of tubes, bottles and plates can be used on the worksurface without compromising throughput, says Rosys. The syringe size is variable between 100 μl and 25 ml. The minimum sample volume in both the alpha and beta models is 5 μl and the maximum sample volumes are 1,500 μl and 250 μl , respectively. The software uses an icon-based Windows 3.0 interface that has multitasking capabilities; this allows even inexperienced users to automate most standard laboratory protocols, says Rosys.

Mega is an **automated assay preparation system** from Tecan, which is designed to provide rapid, high-precision sample preparation for all types of microplate and tube assays (*Reader Service No. 107*). The four liquid-sensitive sampling tips can be configured for use with microplates, standard sample tubes, Eppendorf and microtubes, and up to four different assay preparations can be carried out simultaneously. Alternatively, Mega can be used to perform more complex pipetting protocols, including predilution steps. The Dialogue software menus provide a graphic representation of the



Mega makes light work of automating microplate and tube assays.

work area and facilitate the implementation and storage of up to 50 different assay protocols, each of which can be password-protected to provide multi-user security, says Tecan. Positive sample identification is available using an optional Linear Rack Drive.

Labsystems OY has launched an integrated **EIA management system** called **iEMS**, which can process nine microtitre plates simultaneously (*Reader Service No. 108*). Central to the iEMS, is the microplate analyser, a compact unit that can perform measuring, incubation, mixing and dispensing functions. Plug-in procedure cards and a large display and keyboard ensure fast start up and ease of use. A thermal holder is designed to minimise temperature fluctuations during the assay procedure. Handling of the microplate is also kept to a minimum; it remains in the temperature holder during incubation in the incubator block and during dispensing/reading in the analyser. The iEMS incubator can store up to nine plates and maintains temperatures from ambient to 45 °C. Menu-driven software allows users to create and store up to 100 protocols and perform accurate endpoint, kinetic and agglutination measurements, says Lab-systems.

Filtering aids

Schleicher and Schuell's expanded line of Uniflo disposable syringe filters now includes filters for low-volume filtration and filters with broad chemical resistance (*Reader Service No. 109*). The Uniflo-3 syringe filter, which is designed for filter-

ing volumes of less than 1 ml, uses a 3-mm cellulose acetate filter in either 0.2 µm or 0.45 µm pore size, and achieves low hold-up volumes of less than 15 µl, says S & S. A broad chemical resistance, low protein binding, and low hold-up volumes (<15 µl) are features of the Uniflo-13 syringe filter. The filter, which is made of regenerated cellulose and available in 0.2 µm and 0.45 µm pore sizes, is useful for filtering, volumes of less than 10 mls. Typical uses include the sterilization of media additives and samples, or the clarification of stains and dyes. Completing the line up is the Uniflo-T syringe filter, a 25-mm Teflon



Uniflo syringe filters from S & S.

membrane housed in styrene-acrylonitrile. The filter is designed to provide researchers with a sterile syringe filter that has broad chemical resistance and does not interfere with cell growth.

Bio-Molecular Dynamics has developed a new software-assisted **hollow fibre concentrator/filtrator** that is designed to eliminate some of the problems associated with stirred cells, cross-flow, tangential flow and other pressure filtration devices (*Reader Service No. 110*). The hollow fibre concentrator/filtrator combines the properties of hollow fibres with the simple functions of atmospheric pressure, vertical configuration, gravity and vacuum. The accompanying Electronic Lab Assistant software allows the user to select the optimum conditions for concentration, filtration and dilution-dialysis in order to produce recoveries of up to 99 per cent over a sample volume range of millilitres to litres — independent of macromolecular concentration, says the company.

HPLC: add-on units

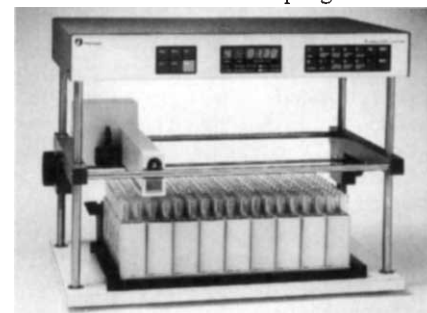
When accessed through the System Gold PC workstation, the Beckman 502 HPLC autosampler is designed to operate unattended with up to 125 different analytical methods (*Reader Service No. 111*). The 502 autosampler can accommodate 92 2-ml vials, with four additional vials reserved for washing the needle. It features filled-loop injection from 10 µl to 2 ml. When controlled by a System Gold workstation, vials can be accessed selectively for more efficient use of sample tray positions. Reproducibility of injection is



Beckman's 502 HPLC autosampler.

less than 0.5 % relative s.d. and carryover is less than 0.2 % with a 50-µl flush, says Beckman. Other options include a built-in programmable column heater and a sample tray heat exchanger that cools labile samples or heats hard-to-dissolve samples in solution. A large sample tray that holds 48 4-ml sample vials and a front-panel keyboard for autosampling applications that are not controlled through the System Gold PC workstation are also available. The basic 502 autosampler costs \$8,800 (US); \$13,100 (US) with all the options.

For applications from laboratory to pilot scale, Pharmacia LKB recommends its **SuperFrac high-capacity preparative LC fraction collector** (*Reader Service No. 112*). Samples can be collected in the microlitre range with collection by volume, drops or time. The fraction collector has a maximum capacity of 312 tubes. Five standard rack types are available for tubes or scintillation vials with diameters of 12–28 mm. A programmable



SuperFrac collects by drops, volume or time. drop head that can be set to 25 different positions allows preparative collection in free-standing vessels of any diameter, says Pharmacia. Up to nine collection programs can be stored and advanced functions such as time-window collection, peak/slope detection, wait and delay, automatic column equilibration, looping, recycling and valve control are designed to add to the sophistication of the instrument. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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